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## A STATISTICAL STUDY OF THE COMPARATIVE MORPHOLOGY OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS<sup>1</sup>

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### INTRODUCTION

Eriksson and Henning (3)<sup>3</sup> and Stakman and Levine (14) have shown that the urediniospores of the various biologic forms of *Puccinia graminis* Pers. differ considerably in size. Stakman and Levine also have shown that the urediniospores of any of the biologic forms become appreciably smaller when the rust is grown on fairly resistant host plants or under other extremely unfavorable environmental conditions.

A mathematical analysis of spore measurements in this connection is of considerable value. Pearl and Surface (7) say, "It is certain that not only are quantitative methods needed in biology, but also that a far more serious need is for something of the methodological viewpoint." Rosenbaum (10) further emphasizes this need by saying "The biometric methods therefore furnish a means of extending the descriptive method and of expressing quantitatively what investigators have heretofore attempted to express qualitatively." This would seem to have a special application in the case of biologic forms, which have always been distinguished on the basis of their physiologic behavior rather than that of their morphologic structure.

The object of this work was to determine by means of statistical studies the morphologic identity, i. e., the limits of variation and the biometric constants for length and width, of the aeciospores, urediniospores, and teliospores of the different biologic forms of stem rust. The studies described in this paper were limited to *Puccinia graminis tritici* Erikss. and Henn., *P. graminis secalis* Erikss. and Henn., *P. graminis avenae* Erikss. and Henn., *P. graminis phleipratensis* (E. and H.) Stak. and Piem., and *P. graminis agrostis* Erikss. It is intended, however, to extend the study to a number of the more important forms of the wheat stem rust, *P. graminis tritici*, which recently have been discovered.

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<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 567.

## EXPERIMENTAL METHODS

The aecial material used for making spore measurements was obtained from several species of barberry (*Berberis* spp.) artificially inoculated with teliospores obtained from wheat, quack grass, oats, and redtop, respectively. The infected plants were collected in the spring of the year at University Farm, St. Paul, Minn. Young, tender leaves of separate barberry plants were inoculated with the teliospores from each of the hosts. Sets of seedlings of wheat, rye, oats, and redtop were inoculated with the mature aeciospores. The infection results thus obtained verified the identity and purity of each of the biologic forms. The aeciospores used for the determination of the spore size of each biologic form were grown under identically the same cultural conditions and on plants of the same barberry species, namely, *Berberis vulgaris* L. In the study of the effect of the host on the dimensions of aeciospores, the cultural conditions were exactly alike, but the barberry species differed in each case. The aeciospores obtained in this manner were first put away as herbarium material and were not measured until several months after maturity. Fifty aeciospores picked out at random from a considerably larger population were measured for both length and width.

Pure strains of urediniospores of each of the biologic forms of *P. graminis* were obtained from specimens of viable uredinial material on grains and grasses, collected at University Farm, St. Paul, Minn., and cultured continuously in the greenhouse on seedlings of different host plants. The measurements in each case were made of healthy urediniospores from the superficial layer of mature uredinia, care being taken to measure spores from uredinia in the same stage of development. In determining the urediniospore dimensions of the individual biologic forms, 200 spores in each population were measured for length and width, respectively, whereas in the study of environmental and host effects 100 spores were measured in every case. For the latter experiment, however, 200 measurements were made of the controls. In the first instance, that is, in the study of the comparative morphology of the urediniospores, the biologic forms concerned were grown on congenial host plants and under uniform and favorable conditions. In the study of relationship of host to urediniospore dimensions, both susceptible and resistant varieties were used, but the environment was maintained uniform and favorable. In the study of the effect of cultural conditions on the size of urediniospores, only one biologic form, *P. graminis tritici*, and one variety of wheat, Haynes Bluestem, were used, the environmental conditions, such as temperature, light, and soil moisture, being interchangeably altered. Control series were conducted simultaneously with the experimental series.

The telial material used in this study was collected at St. Paul, Minn. and kept in the herbarium for nearly a year before the measurements were made. The rust on the common wheat (*Triticum vulgare* Vill.) was *P. graminis tritici*; on the quack grass (*Agropyron repens* (L.) Beauv.) it was *P. graminis secalis*; on the oat (*Avena sativa* L.) it was *P. graminis avenae*; on the timothy (*Phleum pratense* L.) it was *P. graminis phleipratisensis*; and on the redtop (*Agrostis alba* L.) it was *P. graminis agrostidis*. One hundred teliospores were measured in each test and the statistical results computed from these.

All of the spore measurements were made with the same microscope carefully calibrated and standardized. The Zeiss screw micrometer was

used throughout the entire period of the work. The illumination for the microscope was derived from artificial light of uniform intensity. The effect of refraction was eliminated as far as possible by mounting the material in drops of water of approximately equal size. It also was attempted to maintain the quantity of water on the slide nearly constant while the measuring of a given population of spores was in progress.

In order to make the measurements of a given group of spores representative and significant, a sufficient number of measurements must necessarily be made. It was found that in the case of aeciospores a minimum of 50 measurements was required. In the case of urediniospores and teliospores at least 100 measurements were necessary. Greater numbers than these did not seem to add materially to the value of the results obtained in the present investigation, although perhaps they might have been useful in some cases. The longitudinal measurements of teliospores were made from the exterior of the apex to the point of attachment of pedicel, whereas the transverse measurements were made across the septum separating the two cells.

#### COMPARATIVE MORPHOLOGY OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS

The following figures show the dimensions of spores of *Puccinia graminis* and *P. phleipratensis*<sup>4</sup> as given by several different workers, who made their studies at different times.

TABLE I.—Spore dimensions of *P. graminis* and *P. phleipratensis* as given by various authors

Authority.	Range of variation for length and width (in microns).				
	<i>P. graminis</i> .			<i>P. phleipratensis</i> .	
	Aecio-spores.	Urediniospores.	Teliospores.	Urediniospores	Teliospores.
Eriksson and Henning (3, p. 25 and 126)	a 14-26	b 17-40 × 14-22	b 35-60 × 11-22	18-27 × 15-19	38-52 × 14-16
McAlpine (5, p. 121).....	...	20-36 × 14-18	35-63 × 14-25	.....	.....
Plowright (8, p. 162).....	15-25	25-38 × 15-20	35-65 × 15-20	.....	.....
Saccardo (11, v 7, p. 622, and v. II, p. 204).	14-26	24-45 × 14-21	34-60 × 12-22	18-27 × 15-19	35-52 × 14-16
Row (15, p. 695 and 785).	14-26	22-42 × 16-22	35-60 × 12-22	18-30 × 15-20	38-52 × 14-16

<sup>4</sup>The aeciospore measurements are for range of variation in the diameter of these spores.

These dimensions represent the extremes in range of size of two sets of measurements made by the authors.

<sup>5</sup>*P. graminis phleipratensis* (E. & H.) Stak. and Piem. is cited in this instance as *P. phleipratensis* Erikss. & Henn. because the measurements quoted were obtained from sources in which this biologic form of rust was treated as a separate species.

TABLE II.—Frequency of different sizes of 50 aeciospores, 200 urediniospores, and 100 teliospores in each of certain biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform environmental conditions

**[Spores.]**

[illegible]

[illegible]

The differences in the dimensions given by the various workers in the case of *P. graminis* could readily be explained on the basis that more than one biologic form of this species was involved. However, other factors, such as host plant, habitat, personal element, number of measurements made, etc., might have played a part in causing the variation. From Table I, it will be noticed that the size of the urediniospores of timothy rust as given by Sydow differed slightly from those recorded by Eriksson and Henning and by Saccardo. This difference perhaps might be explained as due either to experimental error, variation in the environmental conditions under which the spores were developed, or the number of measurements made. It must be borne in mind, however, that the differences in the dimensions of any of the spores indicated in the table may or may not be really significant. The significance of the difference could not be proved on account of the impossibility of applying a biometric test to the limited data available. In order to determine the value of numerical differences it is necessary to calculate the probable error of these differences and then establish the relationship between the differences and their probable errors. Rietz and Smith (9) and Pearl and Miner (6) state that a difference may be considered as "certainly significant" only when it exceeds its probable error by more than three times, since a difference which is less than three times its probable error "may reasonably be attributed to random sampling" (9), and "that as the ratio, Dev.: P. E., passes 3 the odds against the deviation increase rapidly, reaching a magnitude at 8, which, practically speaking, is beyond any real power of conception" (6).

The biometric constants obtained in this study were calculated according to the methods given by Davenport (2) and Babcock and Clausen (1).

Table II represents a summary of the measurements of all of the biologic forms studied, in their different spore stages, when grown under normal conditions. This table gives the classes according to width and length in microns (each class differing from the next by a single micron), and the number of spores falling into each class both for length and width.

In Tables III and IV, in addition to the variations in the spore measurements, the constants with their probable errors for the different spores of the various biologic forms are given. Table III represents spore lengths while in Table IV spore widths are given. The classes for width differ by one micron ( $\mu$ ) in all cases, whereas the classes for length vary with each spore type. In the measurements of aeciospores the classes differ by 1  $\mu$ , in those of urediniospores by 3  $\mu$ , and in those of teliospores by 5  $\mu$ .

The probable errors of the differences in the statistical results obtained in this study are given in Tables V, VII, and VIII. The bearing they have on the dimensions of the different spore studies is brought out in the discussions of each of the spore types.

In figures 1 to 6, curves are plotted for spore dimensions in microns. These graphs represent the variation in the spore sizes of the biologic forms when the rust was grown on highly susceptible hosts and under very favorable conditions. In each of these graphs the number of spores falling into each class was plotted according to the data contained in Tables III and IV.

TABLE III.—Variations and constants for length of spores of *Puccinia graminis* grown on congenial host plants and under uniform environmental conditions

Spores and biologic forms.	Host plants.	Spore classes according to length.													Total num-ber.	Constants.			
		12 $\mu$	13 $\mu$	14 $\mu$	15 $\mu$	16 $\mu$	17 $\mu$	18 $\mu$	19 $\mu$	20 $\mu$	21 $\mu$	22 $\mu$	23 $\mu$	24 $\mu$		25 $\mu$	Mean.	Standard deviation.	Coefficient of variability.
Aeciospores:																			
<i>P. graminis tritici</i> . . . . .	<i>Berberis vulgaris</i> . . . . .				2	2	2	5	7	18	7	4	1	1	1	50	19.72 $\pm$ 0.19	1.98 $\pm$ 0.13	10.05 $\pm$ 0.68
<i>P. graminis secalis</i> . . . . .	do . . . . .	1	1	2	6	9	11	8	6	4	1	1				50	17.10 $\pm$ .19	2.00 $\pm$ .13	11.70 $\pm$ .79
<i>P. graminis avenae</i> . . . . .	do . . . . .				1	1	6	13	18	9	2					50	18.62 $\pm$ .11	1.20 $\pm$ .08	6.44 $\pm$ .43
<i>P. graminis agrostis</i> . . . . .	do . . . . .	1	2	4	7	14	8	7	4	2	0	1				50	16.46 $\pm$ .18	1.93 $\pm$ .13	11.73 $\pm$ .79
Urediniospores:																			
<i>P. graminis tritici</i> . . . . .	<i>Triticum vulgare</i> . . . . .	15 $\mu$	18 $\mu$	21 $\mu$	24 $\mu$	27 $\mu$	30 $\mu$	33 $\mu$	36 $\mu$	39 $\mu$	42 $\mu$					200	32.40 $\pm$ 0.19	3.89 $\pm$ 0.13	12.00 $\pm$ 0.41
<i>P. graminis secalis</i> . . . . .	<i>Secale cereale</i> . . . . .			8	45	93	43	6	5	14	3					200	27.14 $\pm$ .14	2.91 $\pm$ .10	10.73 $\pm$ .36
<i>P. graminis avenae</i> . . . . .	<i>Avena sativa</i> . . . . .			5	27	75	55	32	6							200	28.50 $\pm$ .15	3.24 $\pm$ .11	11.34 $\pm$ .38
<i>P. graminis phleipra- tensis</i> . . . . .	<i>Phleum pratense</i> . . . . .		7	43	98	50	2									200	23.95 $\pm$ .12	2.41 $\pm$ .08	10.01 $\pm$ .34
<i>P. graminis agrostis</i> . . . . .	<i>Agrostis alba</i> . . . . .	1	23	84	69	22	1									200	22.37 $\pm$ .12	2.61 $\pm$ .09	11.67 $\pm$ .39
Teliospores:																			
<i>P. graminis tritici</i> . . . . .	<i>Triticum vulgare</i> . . . . .	25 $\mu$	30 $\mu$	35 $\mu$	40 $\mu$	45 $\mu$	50 $\mu$	55 $\mu$	60 $\mu$	65 $\mu$	70 $\mu$					100	51.80 $\pm$ 0.49	7.23 $\pm$ 0.34	13.95 $\pm$ 0.67
<i>P. graminis secalis</i> . . . . .	<i>Agropyron repens</i> . . . . .			8	14	30	27	14	6	1						100	47.35 $\pm$ .45	6.65 $\pm$ .32	14.04 $\pm$ .67
<i>P. graminis avenae</i> . . . . .	<i>Avena sativa</i> . . . . .		1	8	18	34	22	13	4							100	46.15 $\pm$ .43	6.46 $\pm$ .31	13.85 $\pm$ .66
<i>P. graminis phleipra- tensis</i> . . . . .	<i>Phleum pratense</i> . . . . .		3	16	43	29	8	1								100	41.30 $\pm$ .32	4.78 $\pm$ .23	11.56 $\pm$ .55
<i>P. graminis agrostis</i> . . . . .	<i>Agrostis alba</i> . . . . .	1	7	24	34	23	9	2								100	40.30 $\pm$ .40	5.87 $\pm$ .28	14.56 $\pm$ .70



TABLE IV.—Variations and constants for width of spores of *Puccinia graminis* grown on congenial host plants and under uniform environmental conditions

Spores and biologic forms.	Host plants.	Spore classes according to width.													Total num-ber.	Constants.				
																Mean.	Standard deviation.	Coefficient of variability.		
		10 $\mu$	11 $\mu$	12 $\mu$	13 $\mu$	14 $\mu$	15 $\mu$	16 $\mu$	17 $\mu$	18 $\mu$	19 $\mu$	20 $\mu$	21 $\mu$	22 $\mu$					23 $\mu$	
Aeciospores:																				
<i>P. graminis tritici</i> .....	<i>Berberis vulgaris</i> .....				2	4	14	21	7	2						50	15.66 $\pm$ 0.10	1.07 $\pm$ 0.07	6.83 $\pm$ 0.46	
<i>P. graminis secalis</i> .....	do.....	2	7	19	13	6	3									50	13.46 $\pm$ .11	1.17 $\pm$ .08	8.69 $\pm$ .58	
<i>P. graminis avenae</i> .....	do.....			1	5	13	22	7	2							50	14.70 $\pm$ .10	1.02 $\pm$ .07	6.97 $\pm$ .47	
<i>P. graminis agrostis</i> .....	do.....	3	12	21	11	3										50	12.98 $\pm$ .09	.97 $\pm$ .07	7.47 $\pm$ .50	
Urediniospores:																				
<i>P. graminis tritici</i> .....	<i>Triticum vulgare</i> .....							2	6	20	44	83	26	15	4	200	19.79 $\pm$ .06	1.27 $\pm$ .04	6.42 $\pm$ .22	
<i>P. graminis secalis</i> .....	<i>Secale cereale</i> .....					3	10	49	60	47	25	5	1			200	17.19 $\pm$ .06	1.26 $\pm$ .04	7.33 $\pm$ .25	
<i>P. graminis avenae</i> .....	<i>Avena sativa</i> .....							3	7	17	38	70	42	17	6	200	19.94 $\pm$ .07	1.36 $\pm$ .05	6.90 $\pm$ .23	
<i>P. graminis phleipratensis</i> .....	<i>Phleum pratense</i> .....				1	5	24	46	58	46	16	4				200	16.88 $\pm$ .06	1.32 $\pm$ .04	7.81 $\pm$ .27	
<i>P. graminis agrostis</i> .....	<i>Agrostis alba</i> .....				4	27	52	72	35	10						200	15.68 $\pm$ .05	1.12 $\pm$ .04	7.16 $\pm$ .24	
Teliospores:																				
<i>P. graminis tritici</i> .....	<i>Triticum vulgare</i> .....			1	3	6	14	20	27	16	6	6	1			100	16.67 $\pm$ .12	1.74 $\pm$ .08	10.42 $\pm$ .46	
<i>P. graminis secalis</i> .....	<i>Agropyron repens</i> .....	1	3	8	10	20	25	18	8	4	3					100	14.77 $\pm$ .12	1.83 $\pm$ .09	12.37 $\pm$ .59	
<i>P. graminis avenae</i> .....	<i>Avena sativa</i> .....			3	6	12	22	25	15	10	4	2	1			100	15.84 $\pm$ .12	1.78 $\pm$ .09	11.24 $\pm$ .54	
<i>P. graminis phleipratensis</i> .....	<i>Phleum pratense</i> .....			2	5	13	25	29	17	7	2					100	15.63 $\pm$ .10	1.46 $\pm$ .07	9.35 $\pm$ .46	
<i>P. graminis agrostis</i> .....	<i>Agrostis alba</i> .....	1	1	9	14	20	28	14	7	4	2					100	14.64 $\pm$ .12	1.72 $\pm$ .08	11.73 $\pm$ .56	

## AECIOSPORES

No previous record of aeciospore measurements of biologic forms of *P. graminis* could be found. The data available relate to the species as a whole, the assumption evidently having been that there was no difference in the size of aeciospores of the various biologic forms. Figures 1 and 2, however, show at a glance the variation existing between different biologic forms at this juncture in their life cycle. The variation is greater in figure 1, in which the curves for spore length are plotted. Here the class containing the greatest number of individuals in the *P. graminis tritici* curve is greater by  $4 \mu$  than the identical class in the *agrostis* curve,

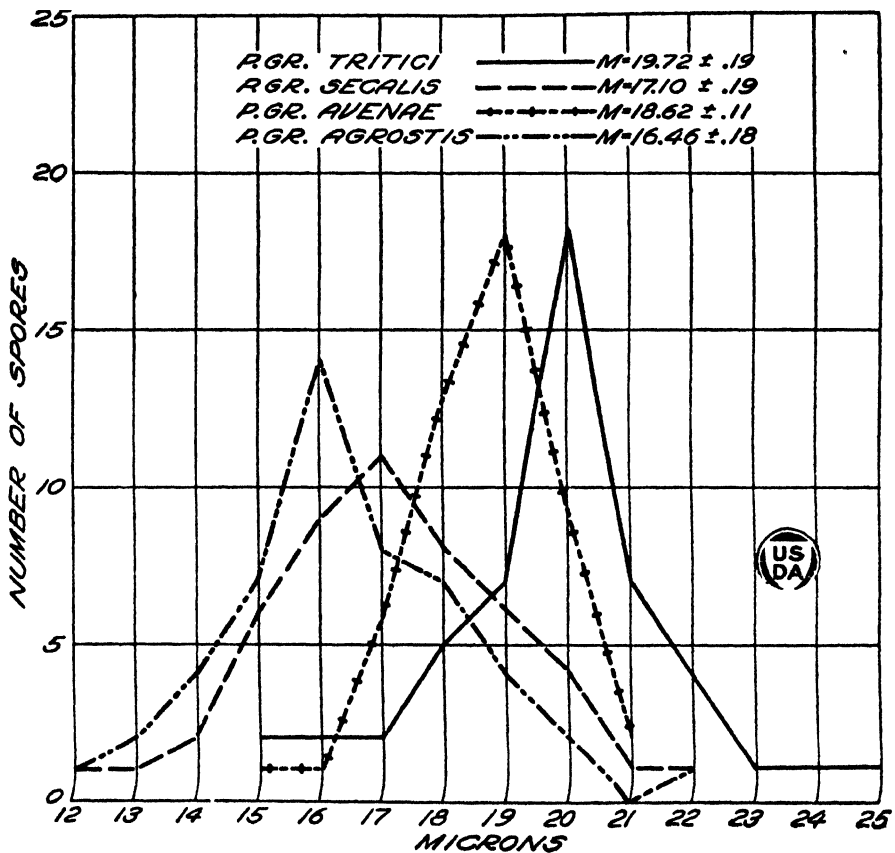


FIG. 1.—Differences in lengths of aeciospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

greater by  $3 \mu$  than that in the *secalis* curve, and greater by only  $1 \mu$  than the homologous class in the *avenae* curve. Figure 2 presents a somewhat different picture. In this graph which represents curves for spore width, the greatest difference between any two classes containing the largest number of individuals is  $3 \mu$ , viz, *P. graminis tritici* and *P. graminis agrostis*. In *P. graminis secalis* and *P. graminis agrostis* the modes fall at exactly the same point, i. e., at  $13 \mu$ . An examination of the constants in the first section of Tables III and IV and of the differences in the means as presented in Table V will reveal not only the existence of a difference in the spore dimensions but in most cases also the distinct significance of such difference.

## P. GRAMINIS TRITICI

The constants, together with their probable errors for the aeciospores of this biologic form, as given in the first section of Tables III and IV, respectively, show this form to have the largest arithmetical mean for both length and width, namely,  $19.72 \pm 0.19 \times 15.66 \pm 0.10 \mu$ .

## P. GRAMINIS SECALIS

The aeciospores of this biologic form differ considerably in both dimensions from those of the tritici form, the means being  $17.10 \pm 0.19$  and  $13.46 \pm 0.11 \mu$ , respectively.

An examination of Table V will show that the difference in the means of these two biologic forms is, without doubt, significant for length as well as for width. The difference in the means of length divided by the probable error of this difference is 9.71, while the difference in the means of the width is 14.66 times greater than its probable error. The odds against the occurrence of such differences in random sampling are beyond comprehension.

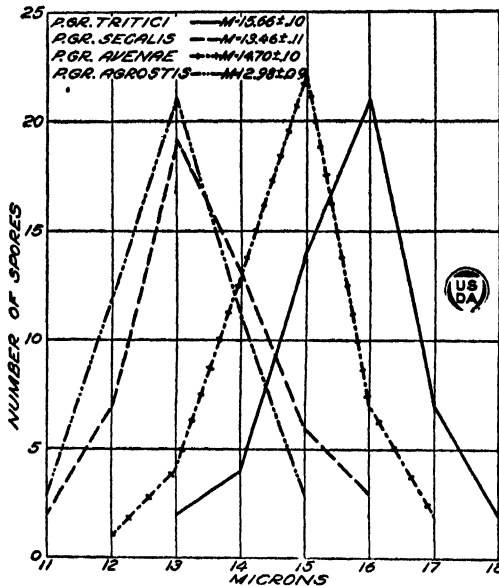


FIG. 2.—Differences in widths of aeciospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

ences, nevertheless, are very significant, as can readily be seen from Table V. The means of the aeciospores of this form are  $18.62 \pm 0.11 \times 14.70 \pm 0.10 \mu$ .

## P. GRAMINIS AVENAE

This biologic form differs quite markedly from either of the above, although to a somewhat lesser extent. The differ-

## P. GRAMINIS PHLEIPRATENSIS

Any attempt to produce aecial infection on different species and varieties of barberry with teliospores of this biologic form has invariably resulted in failure. A study of the aecial stage of this form therefore could not be made.

## P. GRAMINIS AGROSTIS

As shown in Tables III and IV, this biologic form has aeciospores which are smaller in size than those of any of the other biologic forms. The means are  $16.46 \pm 0.18 \times 12.98 \pm 0.09 \mu$ . The differences in the means between this form and tritici on the one hand, and avenae on the other hand, as indicated in Table V, are certainly significant. The difference between agrostis and secalis may or may not be due to random sampling, for the difference in the means of spore length is only 2.46 times greater than its probable error and the difference in width does not exceed 3.43 times the probable error. The odds against the normal occurrence of such differences are approximately 9 to 1 in the length differences, and about 45 to 1 in the width differences. It would be difficult therefore

to consider the differences between the agrostis and secalis forms as truly significant, although they might be considered as indicative. A greater number of measurements might possibly throw more light on this question.

The following table gives the differences in the means of the aeciospores of the four biologic forms discussed in the preceding paragraphs, as well as the ratios between these differences and their probable errors.

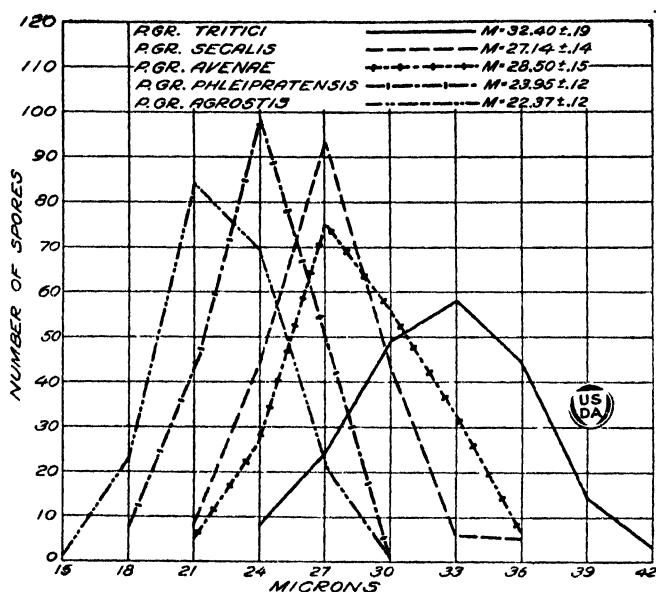


FIG. 3.—Differences in lengths of urediniospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions

TABLE V.—Summary of differences in the means of aeciospores of biologic forms of *P. graminis*

Biologic forms.	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
	Length.	Width.	Length.	Width.
<i>P. graminis tritici</i> and <i>P. graminis secalis</i> .	$2.62 \pm 0.27$	$2.20 \pm 0.15$	9.71	14.66
<i>P. graminis tritici</i> and <i>P. graminis avenae</i>	$1.10 \pm .22$	$.96 \pm .14$	5.00	6.86
<i>P. graminis tritici</i> and <i>P. graminis agrostis</i> .	$3.26 \pm .26$	$2.68 \pm .13$	12.53	20.61
<i>P. graminis secalis</i> and <i>P. graminis avenae</i>	$1.52 \pm .22$	$1.24 \pm .15$	6.91	8.27
<i>P. graminis secalis</i> and <i>P. graminis agrostis</i>	$.64 \pm .26$	$.48 \pm .14$	2.46	3.43
<i>P. graminis avenae</i> and <i>P. graminis agrostis</i>	$2.16 \pm .21$	$1.72 \pm .13$	10.28	13.23

#### UREDINIOSPORES

There is considerably more information found in literature on the relative size of urediniospores of biologic forms of *P. graminis* than on either aeciospore or teliospore dimensions. An interesting phenomenon of spore size relationship becomes apparent when the curves plotted in figures 3 and 4 and the data compiled from previous investigations are examined.

It is that the tritici form possesses the longest urediniospores, the agrostis form the shortest, and the rest group themselves between these two extremes.

TABLE VI.—*Urediniospore dimensions of biologic forms of P. graminis as given by various authors*

Authority.	<i>P. graminis tritici.</i>	<i>P. graminis secalis.</i>	<i>P. graminis avenae.</i>	<i>P. graminis phleipratensis.</i>	<i>P. graminis agrostis.</i>
Eriksson and Henning (3, p. 124 and 130).....	29-43×18-21	23-38×15-22	22-40×16-21	18-27×15-19	.....
Freeman and Johnson (4, p. 27) <sup>1</sup> ..	31-33×18-15	.....	.....	.....	.....
Stakman (12, p. 27) <sup>1</sup> .....	37-85×22-76	.....	.....	.....	.....
Stakman and Jensen (13, p. 215) <sup>2</sup> ..	.....	.....	19-35×16-24	17-31×15-23	.....
Stakman and Levine (14, p. 49) <sup>2</sup> ..	23-42×15-25	18-39×13-21	19-37×14-26	16-32×12-21	15-32×12-20

<sup>1</sup> These figures represent averages, size limits not having been given.

<sup>2</sup> Decimal fractions have been converted to the nearest integer.

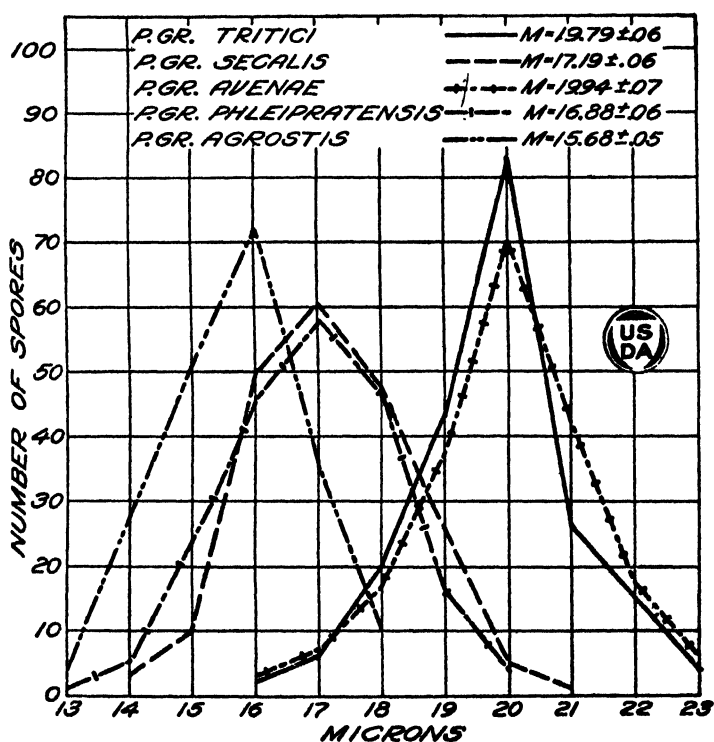


FIG. 4.—Differences in widths of urediniospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

It will also be noted by examining the graphs and the measurements quoted that *P. graminis secalis* and *P. graminis avenae* differ but little in spore length, while the spore width of the latter is much the same as that of *P. graminis tritici*. It will be noted further that according to the graphs as well as the quotations, there is a similarity in the width of the urediniospores of the secalis and phleipratensis forms, but the two diverge noticeably in length. The constants, given in the second section of Tables III and IV, and the differences in the means, presented in

Table VII, bring out not only the variations of the urediniospore dimensions but also the significance of these variations whenever and wherever they are found.

P. GRAMINIS TRITICI

That this biologic form has the longest urediniospores is confirmed by the constants, together with their probable errors, given in the second section of Tables III and IV. In width, the urediniospores are practically the same as those of the oat-rust form. The means for *P. graminis tritici* fall at  $32.40 \pm 0.19 \times 19.79 \pm 0.06\mu$ .

P. GRAMINIS SECALIS

The rye-rust urediniospores are both shorter and narrower than those of the wheat rust as shown by the means,  $27.14 \pm 0.14 \times 17.19 \pm 0.06\mu$ , there being a difference of over  $5\mu$  in length and more than  $3\mu$  in width. There can be no doubt whatever concerning the significance of this difference, as pointed out in Table VII.

P. GRAMINIS AVENAE

Although the urediniospores of this biologic form are only a little more than 1 micron ( $\mu$ ) longer than those of the secalis form, the difference, nevertheless, seems to be quite significant. The means of the urediniospore dimensions of *P. graminis avenae* are  $28.50 \pm 0.15 \times 19.94 \pm 0.07\mu$ .

P. GRAMINIS PHLEIPRATENSIS

The means of this biologic form are  $23.95 \pm 0.12 \times 16.88 \pm 0.06\mu$ . The differences between the means for both width and length of this biologic form and those of *P. graminis tritici* and *P. graminis avenae* are quite significant. The difference in the means, of length only, of the timothy rust and the rye rust is also noteworthy. (Table VII).

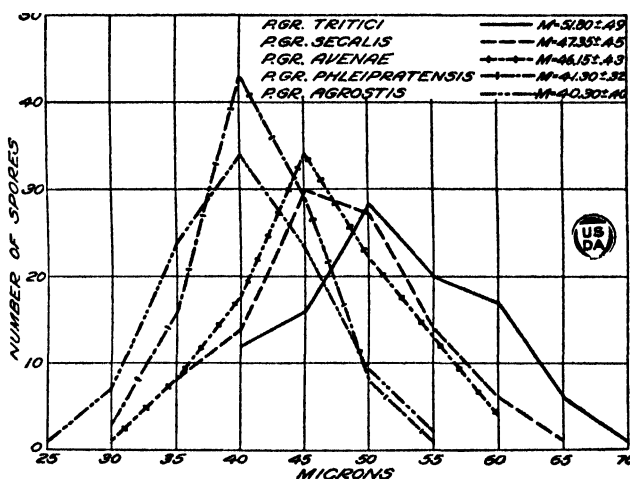
P. GRAMINIS AGROSTIS

The urediniospores of this biologic form are without any doubt smaller than those of any of the other forms. This is demonstrated by the curves plotted in figures 3 and 4 and confirmed by the constants in Tables III and IV, and also by the differences in the means as shown in Table VII. The means of the redtop-rust urediniospores are  $22.37 \pm 0.12 \times 15.68 \pm 0.05\mu$ .

The differences in the means of the urediniospores of the biologic forms of *P. graminis* and the ratios between these differences and their probable errors are summarized in Table VII.

TABLE VII.—Summary of differences in the means of urediniospores of biologic forms of *P. graminis*

Biologic forms.	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
	Length.	Width.	Length.	Width.
<i>P. graminis tritici</i> and <i>P. graminis secalis</i> . . . . .	5. 26±0. 24	2. 60±0. 08	21. 92	32. 50
<i>P. graminis tritici</i> and <i>P. graminis avenae</i> . . . . .	3. 90± . 24	. 15± . 09	16. 25	1. 67
<i>P. graminis tritici</i> and <i>P. graminis phleipratensis</i> . . . . .	8. 45± . 22	2. 91± . 08	38. 38	36. 38
<i>P. graminis tritici</i> and <i>P. graminis agrostis</i> . . . . .	10. 03± . 22	4. 11± . 08	46. 82	51. 38
<i>P. graminis secalis</i> and <i>P. graminis avenae</i> . . . . .	1. 36± . 21	2. 75± . 09	6. 48	30. 56
<i>P. graminis secalis</i> and <i>P. graminis phleipratensis</i> . . . . .	3. 19± . 18	. 31± . 08	17. 72	3. 88
<i>P. graminis secalis</i> and <i>P. graminis agrostis</i> . . . . .	4. 77± . 18	1. 51± . 08	26. 50	18. 88
<i>P. graminis avenae</i> and <i>P. graminis phleipratensis</i> . . . . .	4. 55± . 19	3. 06± . 09	23. 93	34. 00
<i>P. graminis avenae</i> and <i>P. graminis agrostis</i> . . . . .	6. 13± . 19	4. 26± . 09	32. 25	47. 34
<i>P. graminis phleipratensis</i> and <i>P. graminis agrostis</i> . . . . .	1. 58± . 17	1. 20± . 08	9. 39	15. 00

FIG. 5.—Differences in lengths of teliospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

## TELIOAPORES

Published data on teliospore dimensions can be found concerning only one biologic form of stem rust and that is timothy rust. The evident reason is because this form had been considered a distinct species. The other data available refer to the species *P. graminis* as a unit. The various authors consulted agree on the range in the size of teliospores of timothy rust. This is given as  $38-52 \times 14-16\mu$ . Most authors, however, disagree on the size limits of the teliospores of *P. graminis*, perhaps on account of a difference in the biologic forms which had been studied. The curves plotted in figures 5 and 6 point to the existence of a difference in size of the teliospores of the various biologic forms. In general there seems to be a fairly distinct parallellism in the size of the teliospores of the various

forms of *P. graminis* and the size of the urediniospores and aeciospores of the same forms. As in the case of the last two spore types, so in the teliospores, *P. graminis tritici* and *P. graminis agrostis* constitute the extremes with respect to spore size. Figures 5 and 6 show very clearly that the teliospores of the wheat rust are larger in every way than those of any of the other forms. The secalis and avenae forms are practically the same in length but differ noticeably in width. On the other hand, the rusts of rye and of redtop vary insignificantly in spore width. In length the agrostis form approaches that of phleipratensis, but is appreciably different from it in width. The constants given in the third section of Tables III and IV and the differences in the means presented in Table VIII show when these similarities and differences are real and significant and when they are only apparent and inconclusive.

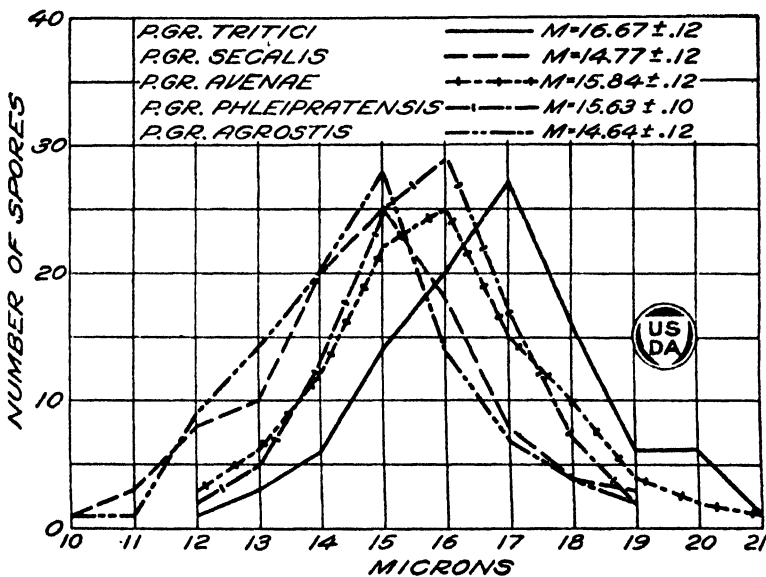


FIG. 6.—Differences in widths of teliospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

#### *P. GRAMINIS TRITICI*

There is a difference of at least 5 microns between the mode for length of the teliospores of this biologic form and the mode for the longitudinal measurement of any other biologic form. A similar difference is found in the means for length. The greatest difference in the width of the teliospore between this and any other form is about one micron for either mode or means. The means for this biologic form, as given in section 3 of Tables III and IV, are  $51.80 \pm 0.49 \times 16.67 \pm 0.12 \mu$ .

#### *P. GRAMINIS SECALIS*

The difference between this biologic form and the one described above can readily be seen by comparing their means, which for the secalis form are  $47.35 \pm 0.45 \times 14.77 \pm 0.12 \mu$ . The difference in the means of the rye-rust and wheat-rust forms is 6.64 times its probable error for length and 11.23 times greater than its probable error for width. Consequently, the difference must be considered as truly significant.



## P. GRAMINIS AVENAE

The means for this form were determined as  $46.15 \pm 0.43 \times 15.84 \pm 0.12 \mu$ . The difference in means between this biologic form and *P. graminis tritici* is obviously quite significant; not so in the case of the difference in the means of the avenae and the secalis forms, however. Here the difference in the means of the length of the biologic forms is only 1.94 times greater than its probable error and therefore insignificant, because such a difference is likely to occur naturally about once in every five random samples. There is, however, a significant difference in the means of the width of these two forms, it being 6.30 times greater than its probable error.

## P. GRAMINIS PHEIPRATENSIS

A reverse condition prevails in the timothy rust, as seen by examining its constants and differences in means. The means of this biologic form fall at  $41.30 \pm 0.32 \times 15.63 \pm 0.10 \mu$ . In other words, the teliospores of this form are considerably shorter than those of the preceding three forms, but of the same or of nearly the same width as the teliospores of the *avenae* form. Table VIII shows that the difference in the means of the width of *P. graminis phleipratensis* and *P. graminis avenae* divided by its probable error is only 1.31, a very insignificant difference. The chances for an occurrence of such a difference are about 38 out of 100.

TABLE VIII.—Summary of differences in the means of the sizes of teliospores of biologic forms of *P. graminis*

Biologic forms.	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
	Length.	Width.	Length.	Width.
<i>P. graminis tritici</i> and <i>P. graminis secalis</i> ..	4.45 $\pm$ 0.67	1.90 $\pm$ 0.17	6.64	11.23
<i>P. graminis tritici</i> and <i>P. graminis avenae</i> ..	5.65 $\pm$ .65	.83 $\pm$ .17	8.69	4.88
<i>P. graminis tritici</i> and <i>P. graminis phleipratensis</i> ..	10.50 $\pm$ .59	1.04 $\pm$ .16	17.80	6.50
<i>P. graminis tritici</i> and <i>P. graminis agrostis</i> ..	11.50 $\pm$ .63	2.03 $\pm$ .17	18.25	11.93
<i>P. graminis secalis</i> and <i>P. graminis avenae</i> ..	1.20 $\pm$ .62	1.07 $\pm$ .17	1.94	6.30
<i>P. graminis secalis</i> and <i>P. graminis phleipratensis</i> ..	6.05 $\pm$ .55	.86 $\pm$ .16	11.00	5.37
<i>P. graminis secalis</i> and <i>P. graminis agrostis</i> ..	7.05 $\pm$ .60	.13 $\pm$ .17	11.74	.77
<i>P. graminis avenae</i> and <i>P. graminis phleipratensis</i> ..	4.85 $\pm$ .54	.21 $\pm$ .16	8.98	1.31
<i>P. graminis avenae</i> and <i>P. graminis agrostis</i> ..	5.85 $\pm$ .59	1.20 $\pm$ .17	9.92	7.06
<i>P. graminis phleipratensis</i> and <i>P. graminis agrostis</i> ..	1.00 $\pm$ .51	.99 $\pm$ .16	1.96	6.18

## P. GRAMINIS AGROSTIS

The redtop rust has the smallest teliospores of any of the biologic forms, although the difference is not direct or absolute. These spores are much shorter than those of either the wheat, rye, or oat rust, but only a little shorter than those of the timothy rust. On the other hand, they are appreciably narrower than those of the wheat, oat, and timothy forms, but much the same in width as the teliospores of the rye rust.

This characteristic variation is brought out more clearly by an analysis of the differences in the means than by a mere consideration of the constants. The means of this biologic form are  $40.30 \pm 0.40 \times 14.64 \pm 0.12 \mu$ . The difference in the means between the agrostis and the tritici forms is significant for both dimensions. Between agrostis and secalis, however, the difference in the means is significant for length only. Again, between the agrostis and avenae forms, the difference is significant for both length and width. The difference in the length of teliospore means of the agrostis and phleipratensis forms has no value as it is only 1.96 times greater than its probable error. The difference in the width, however, appears to be very significant because it exceeds its probable error by 6.18 times.

#### EFFECT OF ENVIRONMENTAL CONDITIONS ON THE MORPHOLOGY OF RUST SPORES

It has been reported (14) that resistant host plants and other unfavorable environmental conditions affecting the normal development and vigor of the rust fungus tend to change appreciably the size of its urediniospores. However, when normal conditions are reestablished, the urediniospores of the following generation are of the original dimensions. This experiment was elaborated and extended to the aeciospores and teliospores. Curves for the distribution of the spores studied were plotted into classes; constants were calculated; and differences in the means of varying conditions established. Tables IX and X represent the spore-size variations for length and width, respectively, as well as the constants together with their probable errors, for the several spore types as affected by different host plants and varying physical factors. The classes in these tables differ in the same order as those in Tables III and IV. The curves in figures 7 to 12 represent the distribution into their respective classes of the spores of the various biologic forms, grown under different conditions. The probable errors of the differences in the means of the spore measurements, together with the ratios obtained from a division of the differences by their probable errors, and the differences in the means themselves are all given in Tables XI and XII.

#### HOST PLANTS

Species and varieties which are congenial to a certain biologic form of *P. graminis* can not, under favorable cultural conditions, exert any perceptible influence on the spore morphology of that biologic form. However, even under identically the same environmental conditions, the spores of any biologic form will be reduced in size when cultured on resistant varieties. This is substantiated by measurements made of spores grown simultaneously on susceptible and resistant hosts.

#### SUSCEPTIBLE HOSTS

Aeciospores of *P. graminis secalis* were produced on *Berberis vulgaris* and *B. sieboldii* Miq., two equally susceptible species. The inoculations were made with teliospores of the same origin. The difference in the size of the two sets of aeciospores was practically negligible and highly insignificant (fig. 7 and 8). The means of the spores on *B. vulgaris* were  $17.10 \pm 0.19 \times 13.46 \pm 0.11 \mu$ , as compared with  $17.44 \pm 0.15 \times 13.30 \pm 0.09 \mu$  for the means on *B. sieboldii*.

TABLE IX.—Variations and constants for length of spores of *Puccinia graminis* grown on different host plants and under varying environmental conditions

Ex- peri- ment No.	Spores and biologic forms.	Host plants or environ- mental conditions.	Spore classes according to length.													Total num- ber.	Constants.		
																	Mean.	Standard deviation.	Coefficient of variability.
			11μ	12μ	13μ	14μ	15μ	16μ	17μ	18μ	19μ	20μ	21μ	22μ					
	Aeciospores:																		
1	<i>P. graminis secalis</i> . . .	<i>Berberis vulgaris</i> . . .		1	1	2	6	9	11	8	6	4	1	1	50	17.10±0.19	2.00±0.13	11.70±0.79	
2	Do. . . . .	<i>Berberis sieboldii</i> . . .				1	5	7	15	9	7	5	1		50	17.44±.15	1.58±.11	9.25±.61	
3	<i>P. graminis agrostis</i> . . .	<i>Berberis vulgaris</i> . . .		1	2	4	7	14	8	7	4	2	0	1	50	16.46±.18	1.93±.13	11.73±.79	
4	Do. . . . .	<i>Berberis brevipani- culata</i> . . .	2	8	11	15	9	5							50	13.72±.12	1.31±.09	9.56±.65	
	Urediniospores:		15μ	18μ	21μ	24μ	27μ	30μ	33μ	36μ	39μ	42μ							
5	<i>P. graminis avenae</i> . . .	<i>Avena sativa</i> . . .			5	27	75	55	32	6					200	28.50±.15	3.24±.11	11.34±.38	
6	Do. . . . .	<i>Bromus tectorum</i> . . .	1	32	43	23	1								100	23.73±.16	2.37±.11	9.97±.48	
7	<i>P. graminis phleipra- tensis</i> . . .	<i>Phleum pratense</i> . . .	7	43	98	50	2								200	23.95±.12	2.41±.08	10.01±.34	
8	Do. . . . .	<i>Dactylis glomerata</i> . . .	1	27	46	26									100	23.91±.15	2.26±.11	9.48±.46	
9	<i>P. graminis tritici</i> . . .	Normal conditions . . .			8	24	49	58	44	14	3				200	32.40±.19	3.89±.13	12.00±.41	
10	Do. . . . .	Excessive heat. . . . .			10	29	40	18	3						100	29.25±.20	2.89±.14	9.88±.68	
11	Do. . . . .	Deficient light . . . . .		1	13	30	37	15	4						100	28.92±.21	3.13±.15	10.76±.51	
12	Do. . . . .	Drought . . . . .			8	19	37	21	14	1					100	30.51±.23	3.47±.17	11.38±.54	
	Teliospores:		35μ	40μ	45μ	50μ	55μ	60μ	65μ	70μ									
13	<i>P. graminis tritici</i> . . .	<i>Triticum vulgare</i> . . .			12	16	28	20	17	6	1				100	51.80±.49	7.23±.34	13.95±.67	
14	Do. . . . .	<i>Triticum dicoccum</i> . . .	3	18	42	27	10								100	46.15±.32	4.79±.23	10.38±.49	

TABLE X.—Variations and constants for width of spores of *Puccinia graminis* grown on different host plants and under varying environmental conditions

Experiment No.	Spores and biologic forms.	Host plants or environmental conditions.	Spore classes according to width.																Total num-ber.	Constants.		
																				Mean.	Standard deviation.	Coefficient of variability.
			10μ	11μ	12μ	13μ	14μ	15μ	16μ	17μ	18μ	19μ	20μ	21μ	22μ	23μ	24μ					
1	Aeciospores:	<i>Berberis vulgaris</i>	2	7	19	13	6	3										50	13.46±0.11	1.17±0.08	8.69±0.58	
2	Do.....	<i>Berberis sieboldii</i>	1	7	23	14	5											50	13.30±.09	.90±.06	6.77±.46	
3	<i>P. graminis agrostis</i> .	<i>Berberis vulgaris</i>	3	12	21	11	3											50	12.98±.09	.97±.07	7.47±.50	
4	Do.....	<i>Berberis brevipaniculata</i> .	2	11	30	6	1											50	11.86±.07	.75±.05	6.26±.42	
5	Urediniospores:	<i>Avena sativa</i> .....						3	7	17	38	70	42	17	6			200	19.94±.07	1.36±.05	6.90±.23	
6	Do.....	<i>Bromus tectorum</i> .						1	10	22	34	29	4					100	18.92±.07	1.08±.05	5.69±.37	
7	<i>P. graminis phleipratensis</i> .	<i>Phleum pratense</i> .....			1	5	24	46	58	46	16	4						200	16.88±.06	1.32±.04	7.81±.27	
8	Do.....	<i>Dactylis glomerata</i> .					2	8	22	33	22	11	2					100	17.06±.08	1.25±.06	7.32±.35	
9	<i>P. graminis tritici</i>	Normal conditions.						2	6	20	44	83	26	15	4			200	19.79±.06	1.27±.04	6.42±.22	
10	Do.....	Excessive heat.						1	6	14	18	35	17	6	2	1		100	19.71±.10	1.45±.07	.7±.35	
11	Do.....	Deficient light.							1	11	20	44	15	8	1			100	19.89±.08	1.13±.05	5.69±.27	
12	Do.....	Drought.....								4	18	36	28	13	1			100	19.31±.07	1.06±.05	5.51±.26	
13	Teliospores:	<i>Triticum vulgare</i> .....		1	3	6	14	20	27	16	6	6	1					100	16.67±.12	1.74±.08	10.42±.46	
14	Do.....	<i>Triticum dicoccum</i> .		1	3	6	16	21	28	13	6	4	2					100	16.57±.12	1.73±.08	10.43±.50	

Similar results were obtained with urediniospores of *P. graminis*. *phleipratensis* of identical origin grown simultaneously on timothy, *Phleum pratense*, and orchard grass, *Dactylis glomerata* L. As can readily be seen from experiments 7 and 8 of Tables IX and X, the spores were alike in length and width; the means for the urediniospores developed on the timothy were  $23.95 \pm 0.12 \times 16.88 \pm 0.06 \mu$ , while the means for the urediniospores obtained on the orchard grass were  $23.91 \pm 0.15 \times 17.06 \pm 0.08 \mu$ . Thus the means in both cases differed only slightly.

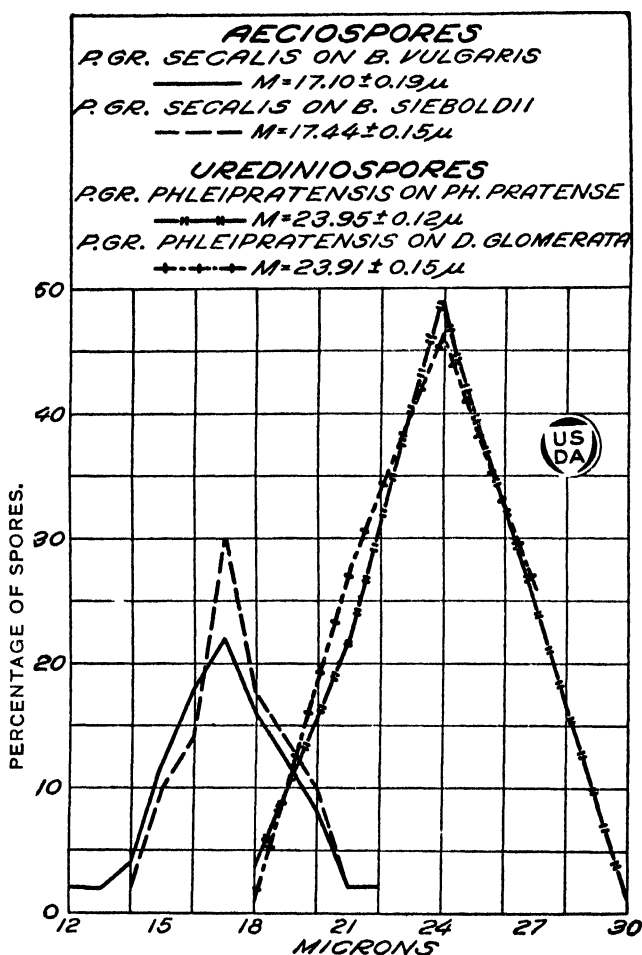


Fig. 7.—Uniformity in length of aeciospores and urediniospores of biologic forms of *Puccinia graminis* grown on different but equally susceptible host plants.

#### RESISTANT HOSTS

As a rule the width of spores is more or less constant under all circumstances. However, a reduction in both length and width as a result of the effect of resistant hosts was obtained in aeciospores of *P. graminis agrostis* produced on *Berberis brevipaniculata* Schneid. and in urediniospores of *P. graminis avenae* grown on *Bromus tectorum*. A reduction in length only occurred in the teliospores of *P. graminis tritici* found on emmer, *Triticum dicoccum* Schr. Figures 9 and 10 illustrate these variations very distinctly.

The aeciospores of the agrostis form on *B. vulgaris* possessed the following means:  $16.46 \pm 0.18 \times 12.98 \pm 0.09 \mu$ , while the means of these aeciospores on *B. brevipaniculata* were  $13.72 \pm 0.12 \times 11.86 \pm 0.07 \mu$ . The difference between the two conditions, as shown in Table XI, is 12.45 times greater than the probable error for length and 10.18 times greater than that for width, which without any doubt is a very significant difference.

*Bromus tectorum* L. produced a similar effect on the urediniospores of *P. graminis avenae*. The means of this biologic form on *Avena sativa*

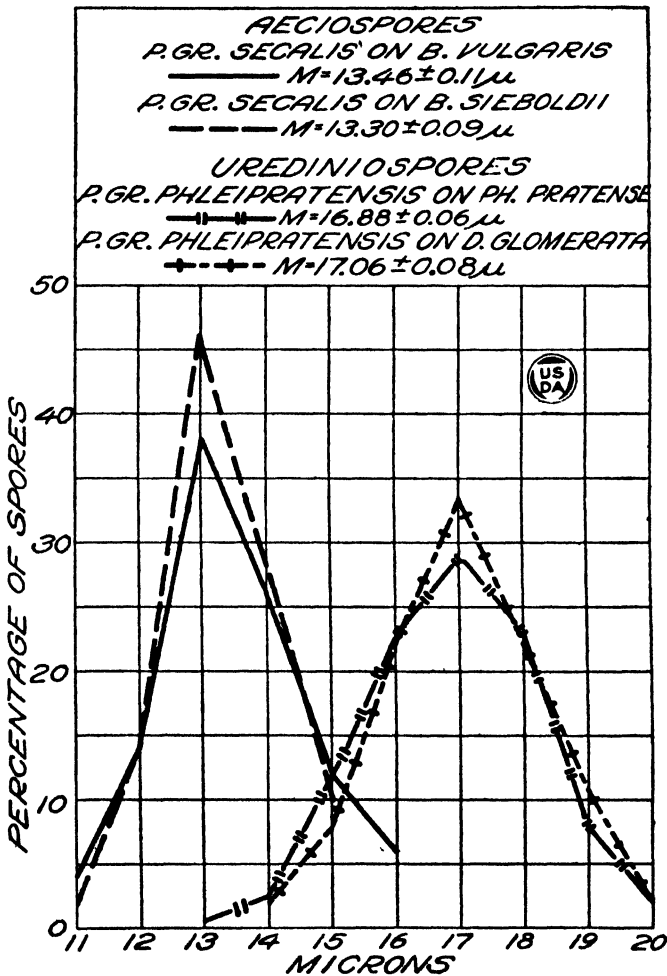


Fig. 8—Uniformity in widths of aeciospores and urediniospores of biologic forms of *Puccinia graminis* grown on different but equally susceptible host plants.

were  $28.50 \pm 0.15 \times 19.94 \pm 0.07 \mu$ , while on *B. tectorum* they were  $23.73 \pm 0.16 \times 18.92 \pm 0.07 \mu$ . The difference in the means of the urediniospores in the two cases was 21.67 times its probable error for length and 10.20 times for width; both are differences as significant as can be desired.

The teliospores on the common wheat were over  $5 \mu$  longer than those on emmer, but practically the same in width. The means for the former were  $51.80 \pm 0.49 \times 16.67 \pm 0.12 \mu$ , while for the latter they were  $46.15 \pm 0.32 \times 16.57 \pm 0.12 \mu$ . The difference in the means of the length only was significant.

Table XI shows the differences in the means of spores of biologic forms of stem rust grown on susceptible and resistant hosts, also the ratios between these differences and their probable errors.

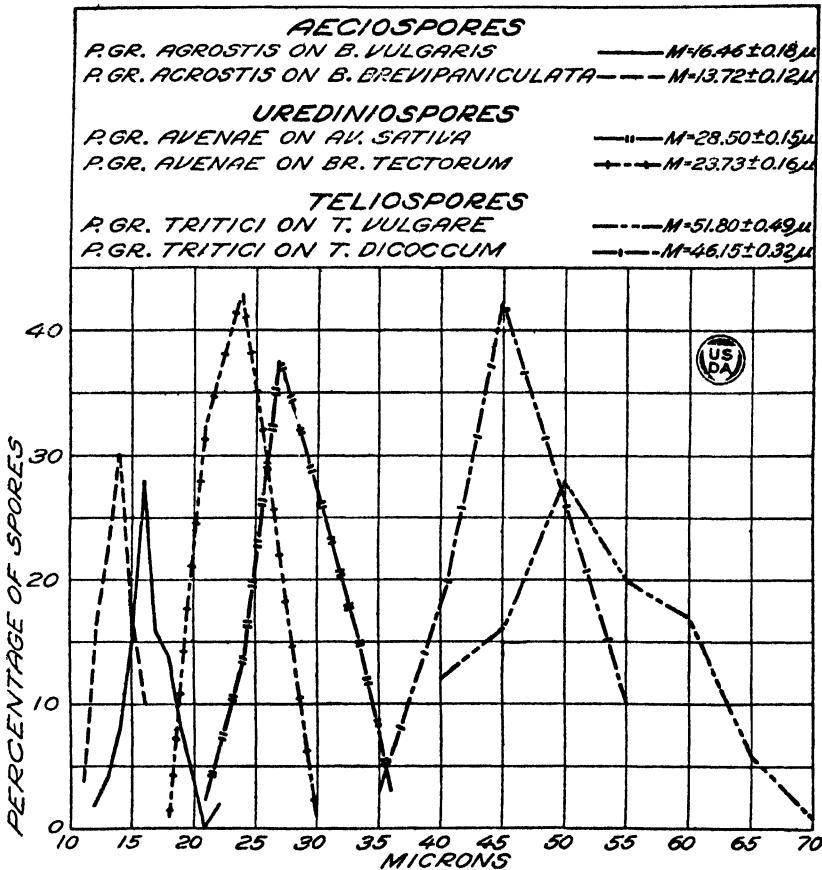


Fig 9.—Differences in lengths of aeciospores, urediniospores, and teliospores of biologic forms of *Puccinia graminis* grown on susceptible and on resistant host plants.

TABLE XI.—Summary of differences in the means of spores of biologic forms of *P. graminis* as affected by various host plants

Biologic forms.	Host plants.	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
		Length.	Width.	Length.	Width.
<i>P. graminis secalis</i> (aeciospores)	<i>Berberis vulgaris</i> and <i>Berberis sieboldii</i>	$0.34 \pm 0.24$	$0.16 \pm 0.14$	1.42	1.14
<i>P. graminis phleipratensis</i> (urediniospores)	<i>Phleum pratense</i> and <i>Dactylis glomerata</i>	$.04 \pm .19$	$.18 \pm .10$	.21	1.80
<i>P. graminis agrostis</i> (aeciospores)	<i>B. vulgaris</i> and <i>B. brevipaniculata</i>	$2.74 \pm .22$	$1.12 \pm .11$	12.45	10.18
<i>P. graminis avenae</i> (urediniospores)	<i>Avena sativa</i> and <i>Bromus tectorum</i>	$4.77 \pm .22$	$1.02 \pm .10$	21.67	10.20
<i>P. graminis tritici</i> (teliospores)	<i>Triticum vulgare</i> and <i>T. dicoccum</i>	$5.65 \pm .59$	$.10 \pm .17$	9.75	.59

## PHYSICAL FACTORS

Unfavorable cultural conditions affect the virulence and spore size of the rust fungus to an appreciable extent, especially the quantity of rust produced and the length of the spores. The inherent nature of infection and the width of the spores are rarely and only slightly changed, if at all. For obvious reasons the effect of environment on the uredinial stage

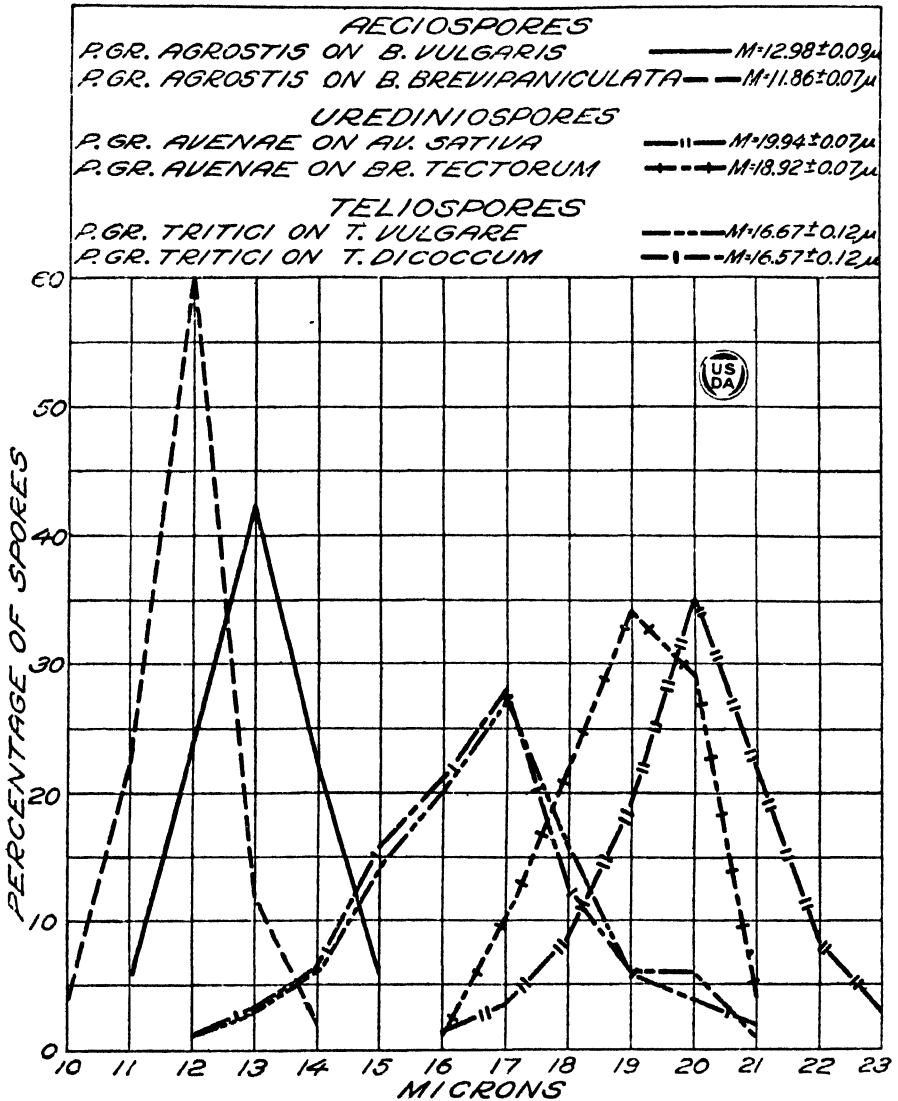


Fig. 10.—Differences in widths of aeciospores, urediniospores, and teliospores of biologic forms of *Puccinia graminis* grown on susceptible and resistant host plants.

alone was studied. Excessive heat more effectively and more conclusively caused the urediniospores of the wheat rust to become shorter than did very low temperature. Deficiency in sunlight and in soil moisture appeared to be equally influential in diminishing the size of the urediniospores. In all of these cases as well as in the control experiment



an extremely susceptible variety of wheat, Haynes Bluestem (Minnesota No. 169), was used as the host plant. Figures 11 and 12 contain curves showing the variations produced by the various conditions.

#### EXCESSIVE HEAT

The means of the spores in the control experiment were  $32.40 \pm 0.19 \times 19.79 \pm 0.06 \mu$ . The means of the urediniospores cultured in high temperature (average for the generation being  $81.8^\circ \text{F}$ . with a maximum daily mean of  $92.3^\circ$  and minimum of  $76.4^\circ$ ) were  $29.25 \pm 0.20 \times 19.71 \pm 0.10 \mu$ . An examination of Table XII shows that the difference in the means of the spore length in the control and temperature experiments was 11.25 times greater than its probable error, whereas the difference in the means of the width was exceeded by its probable error

by 1.5 times; a significant difference for length, but none whatever for width.

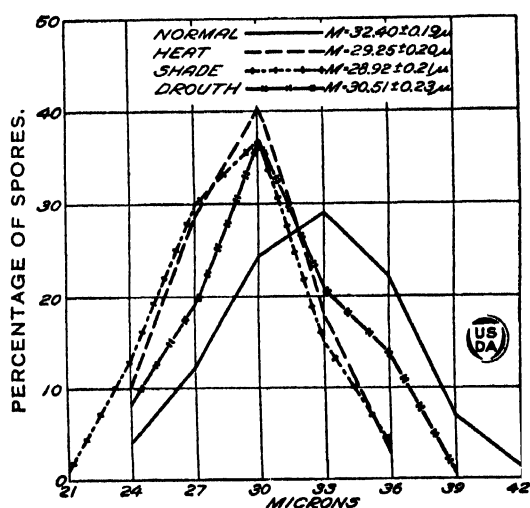


FIG. 11.—Differences in lengths of urediniospores of *Puccinia graminis tritici* cultured under favorable and adverse environmental conditions.

#### DEFICIENT LIGHT

In this experiment the urediniospores were cultured in a light intensity of a total average of 3.1 per cent with a maximum daily limit of 10 per cent and a minimum of 2 per cent. The light intensity readings were made three times a day with the aid of a Clements photometer. The means of the urediniospores in this test were  $28.92 \pm 0.21 \times 19.89 \pm 0.08 \mu$ , which were practically identical with those which prevailed in the

case of excessive heat. Here, too, the difference was significant with respect to spore length only. The difference in the means of the width between the control and shade was exactly the same as its probable error.

#### DROUGHT

The rusted plants in this test received a total amount of 100 cc. of water from the time they were removed from the incubation chamber until the time when the spore measurements were made, 14 days later. The water content of the soil at this time was determined on the basis of the "oven-dried" method and measured 5.38 per cent. The means of the urediniospores subjected to this condition were  $30.51 \pm 0.23 \times 19.31 \pm 0.07 \mu$ . Comparing these means with those of the control spores, we find a difference in both length and width which appears to be significant.

A summary of the differences and their relations to the probable errors of the differences is given in Table XII.

TABLE XII.—Summary of differences in the means of urediniospores of *P. graminis tritici* subjected to various environmental conditions

Environmental conditions favorable except for—	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
	Length.	Width.	Length.	Width.
Excessive heat (average 81.8° F.) . . . . .	3.15 ± 0.28	0.08 ± 0.12	11.25	0.67
Deficient light (average 3.1 per cent) . . . .	3.48 ± .28	.10 ± .10	12.42	1.00
Drought (water content 5.38 per cent) . . .	1.89 ± .30	.48 ± .09	6.30	5.34

## GENERAL DISCUSSION

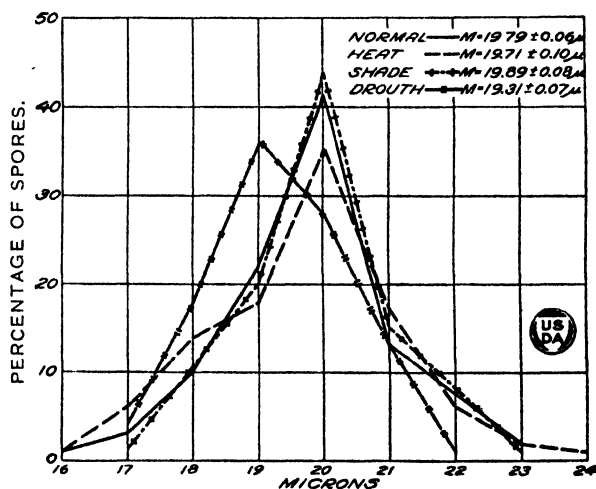
No definite statistical information concerning the dimensions of the different types of spores of the biologic forms of stem rust existed prior to this study.

Measurements of aeciospores, urediniospores, and teliospores, developed under different sets of conditions and on different host plants, were made with the aim of supplying such information. Biometric methods were employed with a view of ascertaining the significance of the variations discovered. These methods were used merely as a means to ascertain more about the nature and behavior and the identity of the different biologic forms. That

results thus obtained are more reliable than a mere mass of individual measurements is now generally conceded.

The statistical data presented in this paper, in addition to corroborating the empirical results obtained by previous workers concerning the differences in the size of urediniospores, also indicate very strongly the existence of similar differences in the aeciospores and teliospores of the biologic forms of *Puccinia graminis*. The differences in magnitude of the various types of spores seem to be quite marked, although they do not occur in any definite order or logical sequence. Sometimes a significant difference appears in both length and width of spores; on other occasions the difference can be detected in the spore length only, the width being almost identically the same, and vice versa.

A numerical difference alone may or may not be significant in itself. A variation of 1 micron in the means of two measurements, for instance, may in one case be ascribed to experimental error and on another occasion constitute a fundamental difference. The difference in the means of teliospores of *P. graminis phleipratensis* and *P. graminis agrostis* is a

FIG. 12.—Differences in widths of urediniospores of *Puccinia graminis tritici* cultured under favorable and adverse environmental conditions.

case which can well illustrate this point. The two biologic forms differ by exactly  $1.00 \pm 0.51 \mu$  in the means of length and by  $0.99 \pm 0.16 \mu$  in the means of width. The difference in length was probably due to random sampling, as it was only 1.96 times greater than its probable error, whereas the difference in width appeared to be rather significant, because it exceeded its probable error by 6.18 times.

Two numerical differences of unequal magnitude, on the other hand, may sometimes be of identical or nearly identical significance. Thus, for instance, the urediniospores of *P. graminis tritici* exceed those of *P. graminis phleipratensis* by  $8.45 \pm 0.22 \mu$  in the means of length, but the difference in the means of width is only  $2.91 \pm 0.08 \mu$ . The difference in the means of length divided by its probable error is 38.38, and that of the width is 36.38. In general, the greater the numerical difference the greater its significance, although in certain cases the situation may be reversed. An excellent illustration of this phenomenon is furnished by the difference in the means of urediniospores of *P. graminis tritici* and *P.*

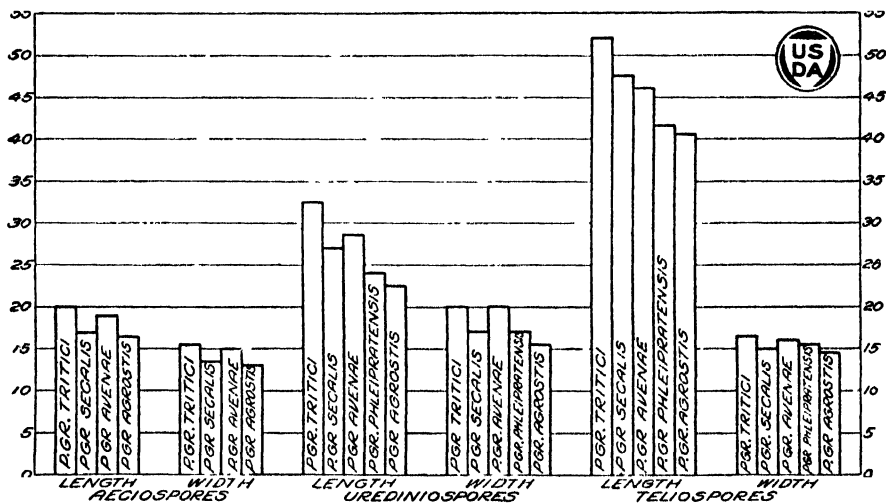


FIG. 13.—Comparisons between the means of lengths and widths of aeciospores, urediniospores, and teliospores, respectively, of biologic forms of *Puccinia graminis*.

*graminis secalis*. The means of the wheat-rust spores were  $5.27 \pm 0.24 \mu$  longer and  $2.60 \pm 0.08 \mu$  wider than those of the urediniospores of the rye rust. But the difference in the means of length exceeded its probable error 21.92 times as compared with 32.50 times for the excess in the case of the difference in width. Thus the important factor in determining the value and significance of differences is not so much the numerical deviation as the probable error of these differences.

The comparative differences in spore dimensions of the various biologic forms are illustrated by photomicrographs and schematic drawings in Plates I and II. Figure 13 represents diagrammatically the apparent correlation between the means of the different forms, while figure 14 shows the rather consistent parallelism in the means of the various spore types.

Although the size of spores is specific for each biologic form, the spores are not rigidly fixed either in size or in shape. Resistant host plants and other adverse environmental conditions, affecting the normal development and vigor of the rust fungus, also affect the size and shape of the

spores. Plate II, F, G, shows the response of the aecial infection of the redtop rust to two species of *Berberis*, one susceptible, *B. vulgaris* (F), and one resistant, *B. brevipaniculata* (G). The effect of these hosts on the size of the aeciospores is graphically illustrated in figures 9 and 10. Urediniospores and teliospores were also affected in a similar manner. This effect, however, is not permanent; it lasts only as long as the unfavorable circumstances prevail. This is especially true in the case of urediniospores. As soon as the rust is transferred to a congenial host plant and favorable cultural conditions are reestablished it again develops normally and the spores attain their normal dimensions.

The ill effect of excessive heat, insufficient light, and deficient soil moisture was quite definitely proved for urediniospores. This would lead to the assumption that aeciospores and teliospores are probably likewise affected by unfavorable cultural conditions.

Host plants belonging to different species and varieties are not in themselves sufficient to cause a change in the normal development of the rust fungus or in the size and shape of the spores unless they vary mark-

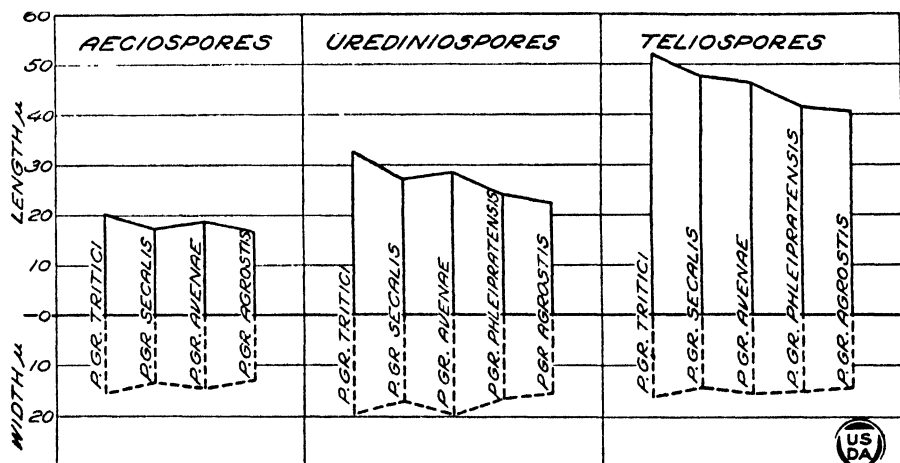


FIG. 14.—Parallelism between the means for lengths and widths of the different spores of biologic forms of *Puccinia graminis*.

edly in the degree of susceptibility to a biologic form. Plate II, D, E, shows an equal amount of aecial infection of *P. graminis secalis* on *B. vulgaris* and *B. sieboldii*. The uniformity in spore size of both cases is illustrated by graphs in figures 7 and 8.

Besides differences in spore size, deviations in the shape of the spores of the various biologic forms were also observed. These were described by Stakman and Levine (14, p. 48) as especially pronounced in the urediniospores. Somewhat less pronounced differences in shape were found by the writer in the aeciospores and teliospores, particularly the latter. Unfortunately the necessary statistical data were not available for calculating the ratios of length to width in the different types of spores, and hence constants could not be computed, nor could differences in mean of diameter be established. This is all the more regrettable because of the full realization of the importance of expressing quantitatively the differences occurring not only in the size but also in the shape of spores. A comprehensive method for this purpose has been described by Rosenbaum (10, p. 250).

## SUMMARY

(1) In studying the nature and behavior of biologic forms careful uniform technic and pure rust strains have been used in order to reduce to a minimum the possibility of experimental error.

(2) Spore measurements can be employed as an additional aid in identifying the biologic forms of *P. graminis*, provided a sufficiently large number of spores are measured for both length and width, and the spores studied are developed on congenial hosts and under favorable conditions. The different measurements should be grouped in classes for length and width, respectively.

(3) On account of the variability in size of the different kinds of spores of each biologic form, a more ready comparison can be made by the use of biometric constants than by a mere mass of individual measurements. By the use of these constants the identity and relationship as well as the nature and behavior of a given biologic form may be further confirmed. Such biometric constants have been calculated and are incorporated in the respective tables.

(4) Numerical differences in spore dimensions may or may not be significant in themselves. The important factor in determining the value of numerical differences consists in the probable error of the differences and in the relation of these differences to their probable errors.

(5) The biologic forms of stem rust differ markedly and significantly in their various kinds of spores when produced under uniform conditions. An interesting parallelism is found to exist between the means of the spore dimensions of the different biologic forms.

(6) In general, the wheat rust, *P. graminis tritici*, has larger spores of each type than any other biologic form. The oat rust, *P. graminis avenae*, occupies the second place; the rye rust, *P. graminis secalis*, the third; and the timothy rust, *P. graminis phleipratensis*, the fourth. The redtop rust, *P. graminis agrostis*, has the smallest spores of all.

(7) The differences in spore sizes do not occur in any consistent direction, nor do they follow a logical sequence. In some cases the spores of two biologic forms may vary in both length and width, in other cases the differences may be in one dimension only.

(8) Congenial hosts do not in any way change the morphology of stem-rust spores. A single host plant, common to several biologic forms, lacks the ability to unify them either in size or shape. Nor can several host plants, which are equally susceptible to a single biologic form, exert any influence on the spore morphology of this form.

(9) Resistant host plants and adverse environmental conditions invariably tend to decrease the size of any type of spores of any biologic form. The reduction in size occurs most frequently in spore length only, but sometimes both length and width are affected. As soon as the unfavorable conditions of host and environment are removed, the spores in question will attain their normal dimensions in a single generation.

(10) It is of interest as well as of importance to establish the quantitative measure of the spore shape of the different biologic forms. For this purpose the ratio of length to width should be determined and grouped into classes, the constants calculated and the significance of the differences ascertained.

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PLATE I

Median longitudinal diagrammatic figures showing comparative differences in the means of aeciospores, urediniospores, and teliospores of biologic forms of *Puccinia graminis*.

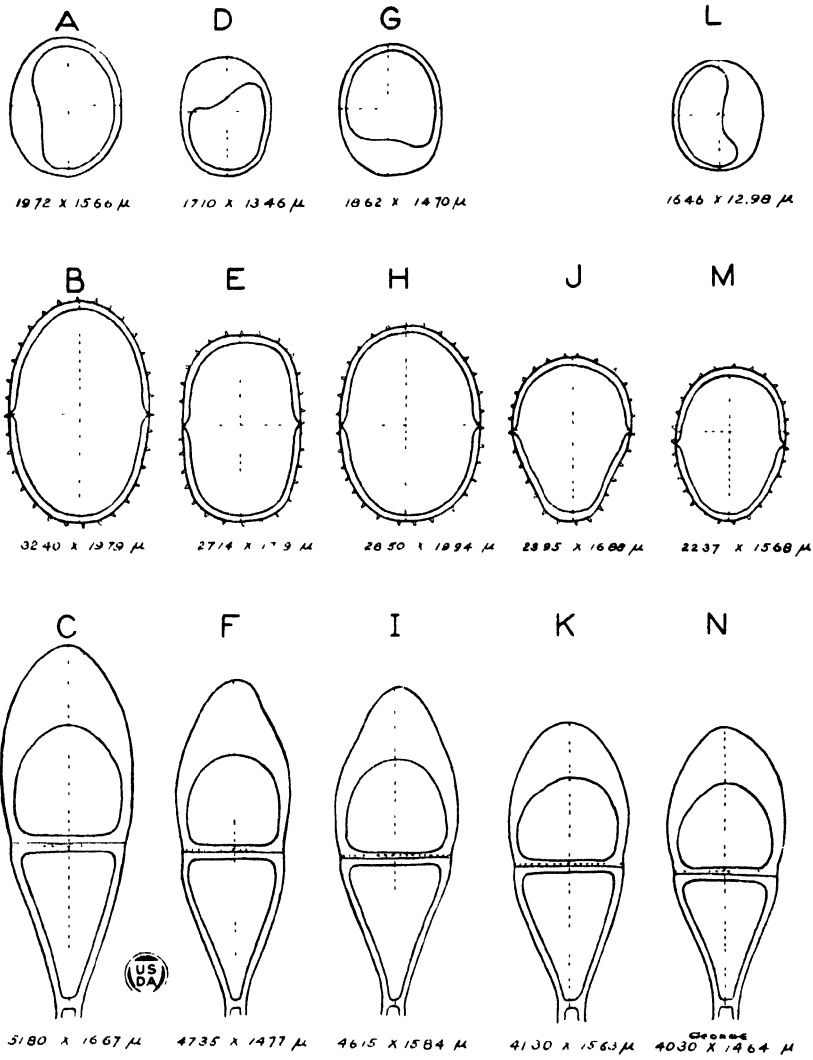
A, B, and C.—Diagrams of aeciospore, urediniospore, and teliospore of *P. graminis tritici*.

D, E, and F.—Similar diagrams of *P. graminis secalis*.

G, H, and I.—Similar diagrams of *P. graminis avenae*.

J and K.—Similar diagrams of urediniospore and teliospore of *P. graminis phleipratensis*.

L, M, and N.—Diagrams of aeciospore, urediniospore, and teliospore of *P. graminis agrostis*.





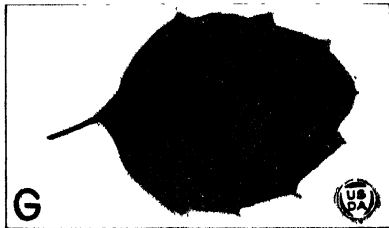
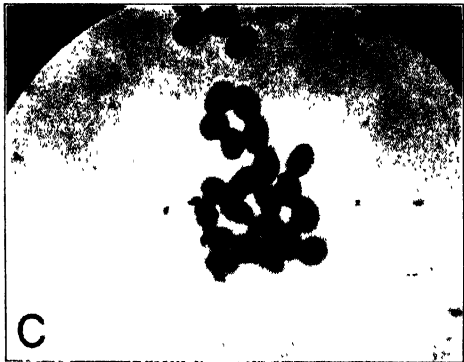
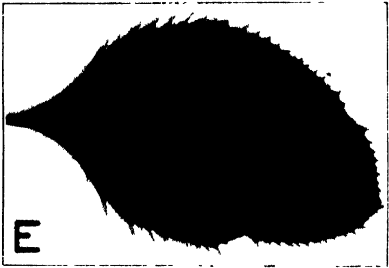
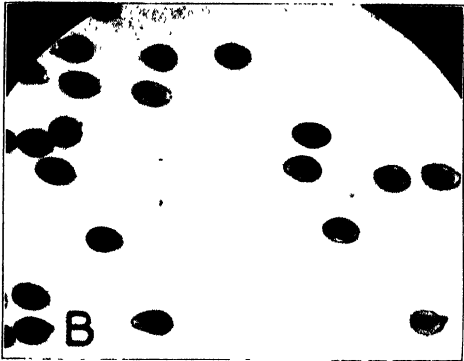
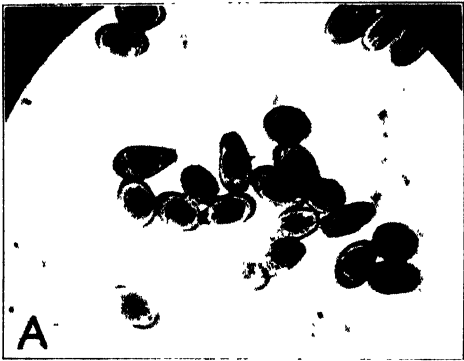


PLATE 2

Photomicrographs of uniform magnification showing the relative size and shape of the urediniospores of three biologic forms of *Puccinia graminis*:

A.—*P. graminis tritici*.

B.—*P. graminis phleipratensis*.

C.—*P. graminis agrostis*.

Photographs illustrating the effect of susceptible and resistant barberry species on the aecial development of biologic forms of stem rust:

D and E.—Showing similar and normal development of cluster cups of *P. graminis secalis* on two equally susceptible species, *Berberis vulgaris* and *B. sieboldii*, respectively.

F and G.—Showing the difference in the aecial development of a single biologic form, *P. graminis agrostis*, when grown on susceptible and resistant barberry species, viz, *B. vulgaris* and *B. brevipaniculata*, respectively.



# RELATION OF CERTAIN SOIL FACTORS TO THE INFESTION OF OATS BY LOOSE SMUT<sup>1</sup>

By LUCILLE K. BARTHOLOMEW, formerly Assistant Pathologist, and EDITH SEYMOUR JONES, formerly Scientific Assistant, Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture<sup>2</sup>

## INTRODUCTION

In order to interpret properly the results obtained by experimenting with a disease, it is desirable to understand the effect of similar conditions upon the host and fungus apart from each other as well as in combination. Accordingly, an attempt has been made in the present paper to analyze the effects of certain soil factors, first upon the fungus, *Ustilago avenae* (Pers.) Jens., and upon the host, *Avena nuda* L., and then upon the two in combination.

The literature relative to the problem in hand has been adequately reviewed in a related publication<sup>3</sup> by the junior author of this paper (in this issue of the Journal of Agricultural Research), so no further detailed consideration of that feature will be attempted in this paper.

## CONDITIONS OF THE EXPERIMENTATION

There are certain environmental factors which must be given thorough consideration in any well-balanced series of pathological investigations. Their relative importance may vary with the host and the fungus under consideration, but their standardization and control, in so far as these are possible, are imperative. Probably the four most important of these factors involved in the present investigations are temperature, moisture, composition of the medium, and its acidic or basic reaction.

The temperatures were regulated very satisfactorily by the use of temperature tanks.<sup>4</sup> The soil moistures were computed and maintained in terms of the saturation point of the soil, which was determined by the commonly accepted standard methods. The soil was sifted through wire gauze into standard 1-centimeter cups, which were leveled off by means of a spatula without tamping, and then were set into a dish of water of known temperature and allowed to stand until the soil was thoroughly saturated. The cups then were removed, drained until no more water dripped from the under surface, and weighed. The soil was dried at a temperature of 100 to 110° C. until it reached a constant weight. From these data the water-holding capacity of the soil was calculated. The standardization of soil water on the basis of dry weight, e. g., 10 gm. of

<sup>1</sup> Accepted for publication Aug. 21, 1922.

<sup>2</sup> The research herein recorded was conducted under the direction of Dr. George M. Reed, formerly pathologist in charge of cereal smut investigations, Bureau of Plant Industry, Washington, D. C., but now at the Brooklyn Botanic Gardens. We wish to extend our grateful thanks to him as well as to Dr. L. R. Jones and his associates in the Department of Plant Pathology, University of Wisconsin, for their hearty cooperation at all times.

This paper presents the results of investigations conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, and the Department of Plant Pathology of the Wisconsin Agricultural Experiment Station.

<sup>3</sup> JONES, Edith Seymour. INFLUENCE OF TEMPERATURE, MOISTURE, AND OXYGEN ON SPORE GERMINATION OF *USTILAGO AVENAE*. In Jour. Agr. Research, v. 24, p. 577-591, 3 fig. Literature cited, p. 590-591.

<sup>4</sup> JONES, L. R. SOIL TEMPERATURES AS A FACTOR IN PHYTOPATHOLOGY. In Plant World, v. 20, p. 229-237, 1917. Literature cited, p. 236-237.

water to 100 gm. of dry soil, was discarded after trial because of the variation in soil-moisture conditions when soils of different water-holding capacities were used. The chief danger of the soil-saturation method lies in the unequal compacting of the soil but this error may be minimized by using a uniform method for handling all soil samples.

The soil moistures were held constant by the use of galvanized containers, insulated soil surfaces, and daily weighings for the determination of any moisture lost through evaporation. The soil reaction was determined by the Truog<sup>6</sup> method and was found in all cases to be "medium acid." The soil composition offered the greatest difficulties in the matter of uniformity, but these were overcome by securing soil from approximately the same locality for all the series of experiments. It was a sandy loam with a water-holding capacity of 28 to 30 per cent for the first year's experiments and 39 to 41 per cent for those of the second year. Pure-line seed of the previous season was used, and sowings were made at a depth of 1 inch. The soil was thoroughly sifted, poured into the can, and tamped by dropping the can from a given height a uniform number of times, thus obtaining a fairly constant state of compactness.

#### INFLUENCE OF TEMPERATURE ON GROWTH OF FUNGUS AND HOST

The fungus, *Ustilago avenae* (Pers.) Jens., was obtained from smutted heads of *Avena nuda* L. grown at Columbia, Mo., in the summer of 1918. This material was used for all of the inoculations during the winter and spring of 1918-19. Smutted heads obtained from our own plats of *A. nuda* L. in the summer of 1919 furnished the inoculum for the following year. Pure-line seed of *A. nuda* L., the hullless or naked oat, was chosen as the standard for experimentation because this species shows a high degree of susceptibility to loose smut. Uniform moistures were maintained throughout the infection period and for one week afterwards, following which the containers were removed from the tanks. The plants taken from the tanks during the colder months were allowed to mature in the containers in which they were growing, but those in the series removed from the tanks in the spring months were transplanted at once to open plats and allowed to mature out of doors.

The study of the fungus in culture has been limited, but the results are clear cut. Potato-dextrose agar, with a reaction of +10 on the Fuller scale, was used. Agar sticks were melted, cooled, and heavily inoculated with sporidia from pure cultures of the fungus. These were poured into plates and allowed to incubate until many colonies appeared. Series of plates of the same medium then were inoculated by selecting uniform colonies and transplanting them. The single-spore method of culturing the organism was found impracticable because of the minute size of the spores, their tendency to cling together, and their poor germination, even when incubated under optimum conditions. The results obtained are shown in Table I.

When the cultures were removed from the Altmann incubators at the end of six days and allowed to remain at room temperature, growth occurred in all except those which had been exposed to 36° C. Growth, however, was very slight in cultures taken from 32°, showing that only a small percentage of the sporidia were able to survive exposure to this temperature. This was not due to desiccation of the medium, because sufficiently high humidity was maintained to prevent undesirable evapo-

TRUOG, E. A NEW TEST FOR SOIL ACIDITY. Wis. Agr. Exp. Sta. Bul. 249, 16 p., 3 fig., 1 col. chart. 1915.

ration. Low temperatures appeared to inhibit growth without causing any permanent injury to the fungus. Cultures were kept at the low temperatures in some cases for a period of four weeks or more, but no growth occurred. When these same cultures were transferred to warmer temperatures, growth soon was evident.

TABLE I.—Effect of temperature on growth of *Ustilago avenae* in culture

Average temperature.	Amount of growth.
C.	
5° .....	None.
8° .....	Very slight.
14° .....	Slight.
16° .....	Fair.
20° .....	Good.
23° .....	Fair.
28° .....	Slight.
32° .....	Very slight (if any).
36° .....	None.

EFFECT OF TEMPERATURE ON GERMINATION OF SPORES AND PRODUCTION OF SPORIDIA

The spores of *Ustilago avenae* placed in Van Tieghem cells in beef broth with a reaction of +10, Fuller's scale, germinated between 5° and 34° C., the minimum being between 4° and 5°, the optimum between 15° and 28°, and the maximum between 31° and 34° C. For sporidial production under the same conditions of culture as for spore germination the minimum and optimum are the same as for spore germination, but the maximum, 30°, is lower.

THE EFFECT OF TEMPERATURE ON GERMINATION OF THE HOST, *AVENA NUDA* L.

It is difficult to estimate the growth of the host as it is not easy to define a standard for growth. The plants in culture, 1 to 2 months old, appeared to be most vigorous where germination and growth of the seedlings had occurred at temperatures of 16° to 24° C. They elongated more rapidly at the higher temperatures but presented an etiolated appearance as compared with the healthy, stalky growth at the lower temperatures. The record is given in Table II.

TABLE II.—Effect of temperature on germination of *Avena nuda* L.

Average temperature.	Observations on growth.
C.	
3° .....	Germination but no subsequent growth.
5° .....	Germination but no subsequent growth.
7° .....	Germination with slight growth.
10° .....	Germination with slow growth.
15° .....	Secondary roots produced, plumule 1.3 cm. long.
19° .....	Optimum growth, plumule 3 cm. long.
21° .....	Good growth, plumule 2 cm. long.
27–33° .....	Less vigorous, plumule 1 cm. long.

Table II gives a fair idea of the effect of the range of temperature, 3° to 33° C., upon germination and seedling growth. The optimum lies at an average temperature of 19° C.

If we compare the host and fungus in their temperature relations in the light of the above data, we find a rather close correlation. The fungus shows a tolerance for somewhat lower temperatures, especially as to germination and initial production of sporidia (essential to infection), while the host shows a tolerance for higher temperatures. However, their optima for germination and growth are practically identical.

#### INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE ON INFECTION

In order to visualize quickly this phase of the problem, it may be well to review briefly the salient points in the life history of the fungus. The spores of *Ustilago avenae*, the loose smut of oats, are for the most part seed-borne. When the oat seed is sown the spores germinate, producing masses of sporidia which continue to bud and multiply much like yeast. As the oat kernel germinates, the sporidia send out germ tubes and infect the young seedling through the coleoptile. By the end of 10 days or 2 weeks the mycelium penetrates through the coleoptile and subsequently establishes itself in the meristematic tissue of the growing point. According to various investigators, infection is limited to the first few days following germination of the oat kernel and none occurs after the growing point emerges from the coleoptile.

As the plant develops, the fungus is carried upward in the growing point of the primary shoot, or of the lateral shoots. Previous to the time of heading, it is impossible to see any difference between smut-free and infected plants, except for a slight lagging in vegetative growth, which is not a safe diagnostic feature. At heading time, however, the fungus appears to gain the ascendancy and the mycelium spreads rapidly through the young ovaries, in which are produced the masses of black spores. At times the fungus appears in linear pustules on the leaves, resembling leaf smut of various grasses, and very often the glumes are striated with the black spore masses. The histological features of the fungus have been adequately described by Lutman.<sup>6</sup>

Obviously the problem in hand is concerned with soil infestation and the physical and chemical conditions of the soil at the time of infection of the oat seedling. Subsequent atmospheric conditions may influence the vegetative growth of the host and thus determine the ability of the fungus to maintain its position in a rapidly dividing meristematic tissue. It is interesting to note in this connection, however, that the most vigorously growing plants appear to be the most heavily smutted. Just why two heads on one plant may be entirely smutted and three other heads on the same plant remain free from smut, or conversely, offers an interesting problem of disease escape. Furthermore, it is not uncommon to see a single spikelet of a single head smutted and all the rest of the head smut-free.

As indicated by the three preceding paragraphs, soil temperature and soil moisture may have a marked influence on infection. Consequently, numerous series of experiments were performed in order to determine the influence of these two factors upon infection of the host by the fungus. The data secured are presented in Tables III and IV.

<sup>6</sup> LUTMAN, R. F. SOME CONTRIBUTIONS TO THE LIFE HISTORY AND CYTOLOGY OF THE SMUTS. In Trans. Wis. Acad. Sci., v. 26, p. 1191-1244, pl. 88-95. 1910. Literature cited, p. 1225-1228.

A uniform soil moisture of 36 per cent (equivalent to 10 grams of water to 100 grams of dry soil with a soil saturation point of 27.8 per cent) was maintained throughout the experiments from which the data in Table III were obtained.

TABLE III.—Effect of different soil temperatures on infection of *Avena nuda* by *Ustilago avenae*, when soil moisture was maintained at 36 per cent

Average temperature.	Dry seed.		Soaked seed.	
	Clean plants.	Smutted plants.	Clean plants.	Smutted plants.
C.	Per cent.	Per cent.	Per cent.	Per cent.
8°	33	67	20	80
12°	50	50	10	90
15°	0	100	0	100
18°	0	100	0	100
21°	0	100	0	100
23°	0	100	25	75
26°	33	67	8	92
29°	25	75	89	11

A soil moisture of 67 per cent (equivalent to 19 grams of water to 100 grams of dry soil) with a soil saturation point of 27.8 per cent was maintained throughout the experiment, the results of which are shown in Table IV.

A comparison of Tables III and IV shows soil temperature to be a limiting factor in infection. The extent of its importance, however, varies with the amount of water present in soil or seed.

TABLE IV.—Effect of different soil temperatures on infection of *Avena nuda* by *Ustilago avenae* when soil moisture was maintained at 67 per cent

Average temperature.	Dry seed.		Soaked seed.	
	Smut-free plants.	Smutted plants.	Smut-free plants.	Smutted plants.
C.	Per cent.	Per cent.	Per cent.	Per cent.
9°	100	0	91	9
12°	47	53	63	37
16°	13	87	72	28
21°	17	83	71	29
26°	39	61	67	33
29°	94	6	100	0

The results given in Table V are based upon the totals secured from a large number of series of experiments and are based on a total of 1,489 plants. The soil moistures are indicated in the table, and the soil-saturation point varies from 39 to 41 per cent. Air-dry seeds were used throughout.

The totals under 80 per cent soil moisture are not based on results obtained from as large a number of plants as in either of the other moisture tests. The high soil moisture was unfavorable for the germination of the grain and many series were reduced or failed utterly as a result, but the percentages given were based on a sufficiently large number to justify inclusion in the results.



TABLE V.—Effect of soil temperature and soil moisture on infection of *Avena nuda* by *Ustilago avenae*

Average temperature.	Soil moisture.	Average smutted plants.	Soil moisture.	Average smutted plants.	Soil moisture.	Average smutted plants.
C.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
10°–14°	30–35	96.5	60	74.0	80	56.0
14°–16°	30–35	96.5	60	58.5	80	1.0
17°–21°	30–35	98.0				
18°–22°	30–35	100.0	60	93.0	(Series failed)	
31°–32°	30–35	24.0	60	0	80	0

When Table V is compared with Tables III and IV, it will be noted that a less pronounced temperature relationship is evidenced, especially at the lower moisture contents. At a temperature of 31° to 32° C. there

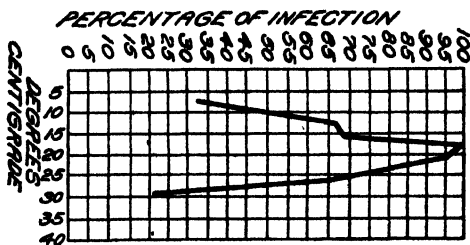


FIG. 1.—Influence of soil temperature upon the infection of *Avena nuda* by *Ustilago avenae* when soil moisture is disregarded.

is a decided falling off in infection at all soil moistures. These results indicate that one would be safe in predicting that high soil temperatures tend to limit infection regardless of soil moistures. When we consider that 32° C. is the maximum temperature for germination and growth of the fungus, we would expect to find this decrease in infection, especially when we consider the

rapidity of germination and elongation of the young seedlings at this temperature.

It will be noted that a temperature of 18° to 22° C. furnishes the optimum for infection in all soil moistures. This coincides directly with the optimum for germination and growth of both host and fungus.

The high percentage of infection at 10° to 14° C. might correlate with the longer time the seed remains in the ground, especially in the case of the lower soil moistures. It may require as long as three weeks under these conditions for the seedling to appear above ground. In this connection, experiments on the relation of soil moisture to the germination of the spores show that the spores germinate as readily in a soil moisture of 35 per cent as in a soil moisture of 65 per cent. If this is correlated with the temperature data already cited, it will be seen that there is no reason why the fungus might not become fairly well established in the soil while the seed remains dormant, or very slowly germinating.

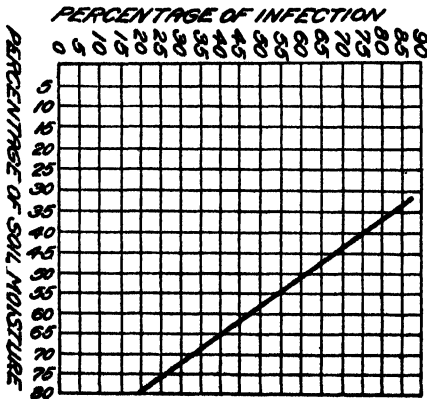


FIG. 2.—The influence of soil moisture upon infection when soil temperature is disregarded.

Such a curve as is shown in figure 1 may help to interpret the common observation that the early sowing of oats in the spring tends to

reduce the amount of infection by loose smut. On the other hand, fall sowing, while the soil temperature is high, would tend also to cut down infection. Such a curve corresponds more nearly to the actual conditions obtaining in nature than a curve derived from series wherein the various factors are considered separately.

The graph in Figure 2 again serves to interpret what is found under field conditions. Conditions of high moisture combined with low temperatures in the spring undoubtedly would tend materially to reduce infection. This conclusion was borne out by some field experiments. Plots containing 28,000 heavily inoculated seeds of *Avena nuda* were prepared in April and May of 1919. The spring was very wet and cold. The average soil temperature was 11.1° C. with a range from 2° to 24° C. during the germination period. The soil moisture averaged from 45 to 50 per cent of the soil saturation point, and ranged as high as 70 per cent at times. The plants matured normally but contained less than one-tenth of 1 per cent of smutted plants. Seed from the same lot treated with the same inoculum, but grown under controlled conditions favorable to maximum infection, produced 100 per cent of smutted plants.

#### SUMMARY

(1) The temperature range for growth of *Ustilago avenae* when cultured on potato-dextrose agar with a +10 reaction is, minimum 8° C.; optimum 20° C.; maximum 32° C.

(2) The temperature range for germination of the spores in beef broth with a +10 reaction is, minimum 5° C.; optimum 15° to 28° C.; maximum 31° to 34° C.

(3) The temperature range for sporidial production under the same conditions as for spore germination is, minimum 5° C.; optimum 15° to 28° C.; maximum 30° C.

(4) The temperature range for germination of the seeds of *Avena nuda* is, minimum 3° C. with no measurable subsequent growth; optimum 18° to 20° C.; maximum 33° C. plus, not definitely established.

(5) The temperature at which the seed is germinated and the seedlings maintained for two weeks appears to influence the subsequent vigor of the plants. The most vigorous plants were secured from seedlings which had been kept at 16° to 24° C.

(6) High soil temperatures were accompanied by a marked reduction in the percentage of infection under the conditions of our experiments.

(7) Low soil temperatures also were accompanied by a reduction in the percentages of infection, but to a much less marked degree than in the case of high temperatures.

(8) High soil moistures, combined with high soil temperatures, result in complete elimination of the fungus.

(9) Low soil moistures, within a certain temperature range, are accompanied by relatively high percentages of smut infection.



# INFLUENCE OF TEMPERATURE, MOISTURE, AND OXYGEN ON SPORE GERMINATION OF *USTILAGO AVENAE*<sup>1</sup>

By EDITH SEYMOUR JONES, *Formerly Scientific Assistant, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

## INTRODUCTION

The relation of climatic factors to the development of the smuts has been a matter of speculation during rather more than two decades. An exact determination of the influence of these factors has recently been undertaken by Dr. Lucille K. Bartholomew and the writer (2),<sup>2</sup> in order to provide experimental conditions for the determination of the smut resistance of certain pure lines of species of *Avena*. It has been pointed out in that paper that in order to decide whether any given variety be completely resistant, its performance under conditions resulting in 100 per cent of smut infection in susceptible plants must be ascertained. In determining those factors most favorable to the development of the smut organism, it becomes manifest that these same factors are numerous and closely interwoven, affecting both the host and the fungus. Their effect on both the host and the fungus must be distinguished if a complete analysis of their relation to the development of the disease is to be achieved.

While Doctor Bartholomew and the writer were determining the effect of temperature and moisture (2) upon infection by *Ustilago avenae* (Pers.) Jens. (5), the writer began to study the influence of temperature upon the germination of the spores of this fungus. When the results of the infection studies began to indicate that soil moisture is a factor controlling infection, the effect of this factor upon the germination of the spores was studied, and later the necessity of studying the relation of oxygen to spore germination became evident. This paper presents the experimental evidence which has been obtained concerning the influence of these three factors upon the germination of the spores of *Ustilago avenae*. Most of these experiments were performed under the guidance and with the help of Doctor Bartholomew, who had carefully formulated the whole problem in its broader aspects.

By way of further introduction to the review of literature and the experiments, it may be well to state briefly the life history of the fungus in its relation to the infection of the host plant. The spores of *Ustilago avenae* overwinter mainly on the seed. The spores germinate simultaneously with the oat seed. They rarely form germ tubes but commonly produce promycelia which, in turn, form either sporidia or thin hyphæ. These sporidia, hyphæ, or germ tubes infect the host through the coleoptile before it is pierced by the first leaf and the fungus establishes itself in the meristematic tissue of the growing point of the main axis and the tillers. Here the mycelium keeps pace with the development of the host, without apparent injury to it, until, at blossoming time, it

<sup>1</sup> Accepted for publication Aug. 27, 1923. This paper presents the results of investigations conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, and the Department of Plant Pathology of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> Reference is by number (italic) to "Literature cited," p. 590-591.

produces its chlamydospores in the young ovaries. Many of these spores, when scattered, come in contact with the seed and the life cycle is repeated.

## REVIEW OF LITERATURE

### RECOGNITION OF THE RELATION OF CLIMATE TO THE SMUTS

The first recognition of a relation between environment and the development of the smuts of oats, or, in fact, of any closely related smut, was made by Jones (15). The observations recorded in that publication indicated, in his opinion, that certain climatic conditions prevailed in Vermont which were especially unfavorable for the development of oat smut. In the following year, however, Jones (16) found a higher percentage of smut in Vermont than in other sections of the country. These observations were supplemented by the records, for one season, of the soil temperature during the period of germination and early development of the seedling. From these accumulated data, the writer concludes—

it is evident that surrounding climatic or soil conditions may at any point in the contest so favor one or the other of the contestants—either the oat plant or the smut fungus—as to decide which shall come out victorious.

### ANALYSIS OF THE RELATION OF TEMPERATURE TO THE SMUTS

Following Jones, other workers have recognized soil temperature as a factor of primary importance. Among them Hecke (12) distinguishes a threefold effect of temperature, involving particularly (1) the germination of the spores and seeds, (2) the duration of conditions in host plant favorable for infection, and (3) the possibility of the fungus reaching the growing point of the plant. The greater number of the other publications dealing with the relation of temperature to the disease can be classified according to the aspects thus distinguished.

### EFFECT OF TEMPERATURE UPON GERMINATION OF SPORES AND SEEDS

Herzberg (13) found the cardinal points of temperature for spore germination of *Ustilago avenae* and of four other smuts to be as follows: Minimum between 5° and 11° C., optimum 22° to 30°, and maximum 30° to 35°.

Ravn (20) very early noted that the amount of oat smut varies with the time of sowing oats. Later, detailed experiments made possible the definite statement that the time of sowing affects greatly the occurrence of oat smut, that the quantity of smut is small or negligible when oats are sown early, and that it increases in later sowings. A decrease with very late sowing was observed in a few cases. In his discussion of the possible reasons for this climatic influence he presumes that the temperature during germination is the most important factor.

Tubeuf (22), experimenting with the smut of oats, found no infection when the temperature was below 7° C. during the seedling stage and 20 to 24 per cent at a temperature of 20° to 21°. In accounting for this difference, he showed that the minimum for the germination of the oat plant is lower than that of the smut, so that when the temperature is sufficiently low the host will escape infection. Thus, he is led to recommend early sowing. In a short paper, Appel and Gassner (1) make some general remarks on oat smut, which are essentially in agreement

with the views of Tubeuf (22), as given in the first of this paragraph. Also Eriksson (7) considers that damp, warm weather during the sowing favors infection. Heald and Woolman (10) have found that a range of temperature between 40° and 65° F. during the germination of wheat is more favorable for bunt infection than either a higher or a lower temperature. It is of interest that this range includes the minimum and the optimum for the germination of bunt spores as given by Hecke (12). There are several nontechnical bulletins which mention the importance of temperature. Heald (9), in advising the early or late sowing of wheat, shows that during August not only is there a comparatively small number of smut spores in the soil, but that also the soil temperature is generally too high for optimum infection; and by the end of October or the first of November, not only has much of the wind-blown smut germinated, but also there is the frequent occurrence of a soil temperature too low for ready infection. In a similar publication, Heald and Zundel (11) state that early seeding of oats results in the minimum amount of smut.

#### DURATION OF SUSCEPTIBILITY OF HOST PLANT TO INFECTION

Volkart (23) was the first to emphasize the fact that low temperature may prolong the infection period. He states that with oats and wheat the slower the initial stages of growth, the more abundant and severe will be the infection. However, extremely low temperatures may retard not only the germination of the seed but also may retard even more the germination of the smut spores and thus remove the danger of infection. This point is more clearly set forth by Hecke (12). The latter's experiments dealt with the bunt of wheat which he found occurred most abundantly among plants which had passed their early stages during the low temperatures of late fall and early spring. The temperature of the first five days seemed decisive. That low temperatures at that time caused increased infection he lays in the main to the prolonging of the infection period, although he also discusses what he considers a greater ease with which the fungus may reach the growing point. In comparing oat smut with bunt in this temperature relation, he considers the low temperature not only as a factor retarding the development of the oat seedling, but also as one delaying the germination of the smut spore. The possibility of this being true is substantiated by the cardinal points of temperature. These, according to his citations, are as follows: For oats, minimum 4° to 5° C., optimum 25°, and maximum 30°; for oat smut, minimum 5° to 11° (9°), optimum 22° to 30°, maximum 30° to 35°; for wheat, minimum 3° to 4.5°, optimum 25°, maximum 30° to 32°; and for bunt spores, minimum less than 5°, optimum 16° to 18°, maximum less than 25°.

Munerati (19) emphasizes the importance of the duration of the infection period for heavy bunt infection. He states:

The faster the first phases in the evolution of the life of the seedling are passed through, the more completely is it able to escape attack by *Tilletia*, and vice versa.

He comes to such conclusions as the result of experiments recorded in two publications (18, 19).

It is obvious that a deeper sowing of seeds might have the effect of prolonging the infection period in a way similar to a low temperature. Jones (16) makes note of the factor of the depth of seeding.

Kirchner's (17) conception is at variance with that of Volkart (23), Hecke (12), and Munerati (19). Kirchner, also working with bunt, says

there is no connection between the ability of the young plant to push through the ground and the attack of smut. He considers the susceptibility of the host to be dependent upon internal chemical differences, and that the host is not affected by external conditions so as to permit the entrance or bring about exclusion of the smut.

#### POSSIBLE EFFECT OF TEMPERATURE ON THE ABILITY OF THE FUNGUS TO REACH THE GROWING POINT OF THE HOST AFTER PENETRATION

Hecke's third aspect, the possibility of the fungus reaching the growing point of the host plant, is difficult to distinguish from the second, the prolongation of the infection period. Investigators dealing with the disease do not know without studying the host and the fungus separately which of these is modified by the temperature factor. Hecke classes Brefeld's (3) work under the third aspect in which the fungus is considered modified. Using germinated oat smut spores as inoculum, with a lower temperature (one not over 7° C.) he obtained 40 to 46 per cent infection, whereas at a higher temperature (above 15° C.) he obtained 27 to 30 per cent. With the factor of the germination of the smut spores eliminated, Hecke concluded that the fungus reached the growing point of the host more easily at the lower than at the higher temperature. From his results, Brefeld considers that if, in cultivation, the seeding is done in warm weather, followed by cold, more smut will develop than when the temperature remains constantly high. The use of germinated spores as inoculum makes it impossible to compare Brefeld's work with that of other investigators of oat smut.

#### RELATION OF MOISTURE TO THE SMUTS

The relation of moisture is discussed by Clinton (4) and by Hungerford and Wade (14). The former notes greater loss from both loose and covered smut of oats in the "hardpan region" of Illinois than in central Illinois. He thinks that the higher moisture content of the soil in this region favors the fungus and causes more infection. The latter observed that in fields sown immediately after rain, a higher percentage of bunt appeared than in those sown under relatively dry conditions. Results obtained from their greenhouse experiments seemed to confirm this observation.

#### EXPERIMENTAL STUDIES

##### THE INFLUENCE OF TEMPERATURE ON SPORE GERMINATION

The first problem was to redetermine the cardinal points of temperature for spore germination. The spore material used was grown the spring previous to experimentation and was from 2 to 7 months old when used. To insure its uniformity, all the smutted heads were kept in one envelop, and the spores shaken from the heads were introduced into 30 cc. of medium in approximately the same quantities each time. After trying several different media, a beef broth (4 gm. Liebig beef extract and 10 gm. peptone per 1,000 cc. of distilled water) of +10 Fuller's scale ( $P_{H6.1}$ ) was chosen. About two small loopfuls of the spore suspension were mounted in Van Tieghem cells or hollow-ground slides. As Duggar (6) has shown the form of the drop and the amount of evaporation to be significant, care was taken that all conditions of mounting

should be as uniform as possible. Duplicate mounts were placed at each temperature, and the experiment was replicated 15 times. Automatically regulated incubators furnished the constant temperatures to which the spores were subjected. At each observation the percentage of germination of 400 spores was recorded for each cell.

The results of a typical experiment are expressed in Table I, and Figures 1 and 2. The results of all the experiments are brought together in Table II.

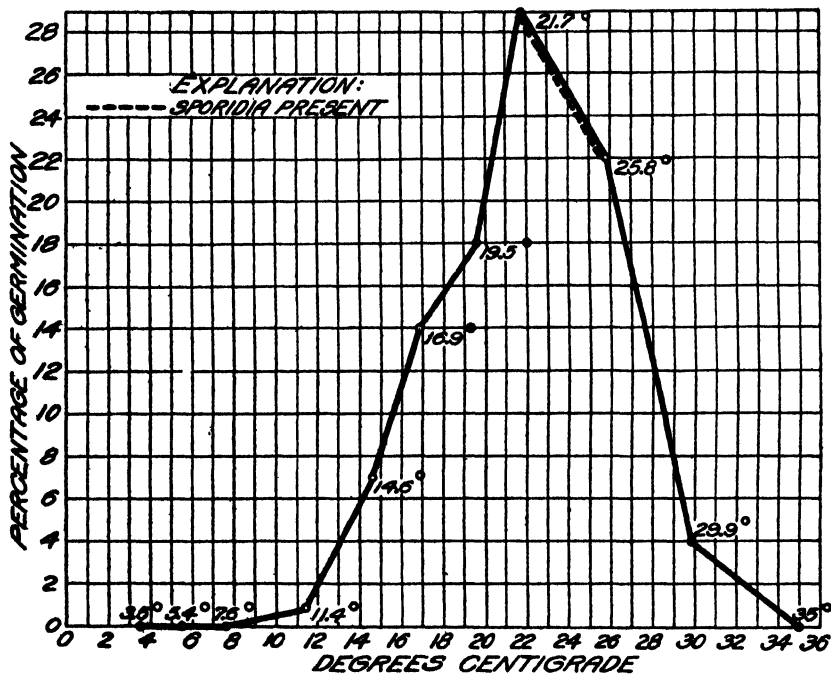


FIG. 1.—Average percentage of germination, and presence of sporidia, of *Ustilago avenae*, in 24 hours, as influenced by temperature of incubation, in experiment 5.

TABLE I.—Average percentage of germination of chlamydospores of *Ustilago avenae* produced 5 months earlier, incubated at different temperatures, and recorded at successive intervals

Temperature average.	Temperature range.	Time.									
		¼	1	2	3	4	5	6	7	8	9
		Days.	Days.	Days.	Days.	Days.	Days.	Days.	Days.	Days.	Days.
C.	C.										
3.5°	2.6°-4.8°	0	0	0	0	0	0	0	0	0	0
5.4°	4.4°-6.0°	0	0	0	0	0	.4	2	3	8	10
7.6°	6.4°-8.8°	0	0	0	2	14	18.0				
11.4°	10.0°-12.5°	0	.7	10	27	27					
14.6°	13.6°-15.9°	0	7.0	22	27	34					
16.9°	16.1°-18.5°	0	14.0	21	32						
19.5°	17.8°-20.6°	.7	18.0	27	27						
21.7°	19.7°-23.5°	3.0	29.0	27	31						
25.8°	22.5°-28.4°	1.3	22.0	33							
29.9°	28.4°-31.4°	.5	4.0	2	5	3					
35.0°	34.0°-36.0°	0	0	0	0	0					



In order that Table II be clear to the reader, there are four facts which must be borne in mind. First, in many cases the incubators did not hold the temperatures within satisfactory limits, so that the upper limit of the temperature range was more significant than the average temperature, especially when the spores were incubated for a long time. For this reason the minimum temperature for spore germination rests between  $4^{\circ}$  and  $5^{\circ}$  C. In the second place, the age of the spore material influences very markedly the percentage of germination and, to a small degree, extends the range of temperature within which the spores will germinate. This is seen clearly in the germination between  $31.7^{\circ}$  and  $32.7^{\circ}$  C. Of six observations made after 6 hours, germination occurred when the spore material was 2 and 4 months old, but not when it was 5 and 6

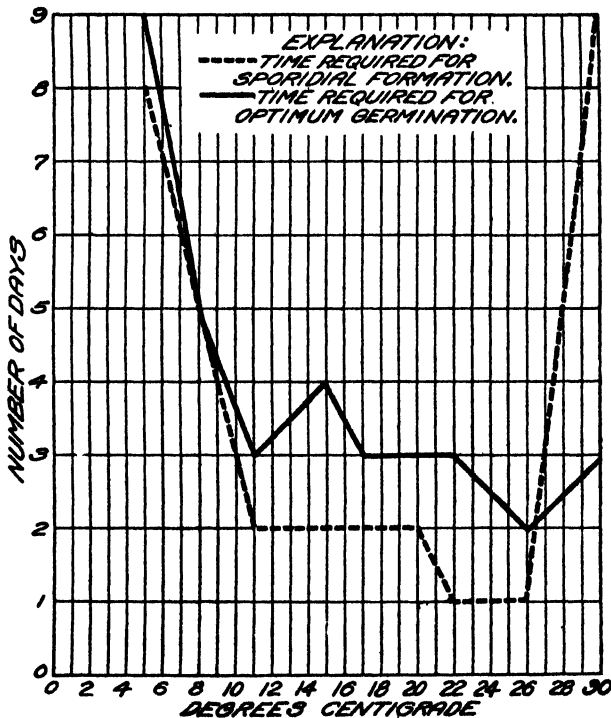


FIG. 2.—Time required for maximum percentage of germination and sporidial formation with *Ustilago avenae*, at temperatures ranging from  $4^{\circ}$  to  $35^{\circ}$  C., in experiment 5.

months old. Third, the spore material used for experimentation in the second year showed a higher percentage of germination, and germination at a wider range of temperatures than that used the first year. This accounts for the fact that experiments 11 to 15, inclusive, conducted during the second year, show higher percentages than the earlier experiments. In the fourth place, germination apparently decreased after a certain length of time, as some of the figures in the table show. This, of course, could not be due to an actual decrease, but was due to the fact that the promycelia broke away from some of the spores, leaving them indistinguishable from ungerminated spores. The examinations were discontinued in most cases when lower percentages were obtained. Where the records were made, however, the figures were recorded to show in a more decisive way where the maximum germination was attained.

TABLE II.—Results of experiments 5 to 10, conducted in 1918, and of experiments 11 to 15, conducted in 1919, showing the average percentage of germination of the chlamydospores of *Ustilago avenae* of different ages, incubated at different temperatures, and recorded at successive intervals.

Exper- iment No.	Age of spores.	Average tem- pera- ture. C.	Temperature range.	Time in days.																			
				1/4	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	19	20	28
	Months.	C.	C.																				
8	6	2.0	2.4-3.80	0			0	0			0										0		
9	7	2.90	2.4-3.80	0			0	0			0												
12	2	3.50	2.6-4.80	0			0	0			0												
5	3	3.50	2.9-4.40	0			0	0			0												
7	5	3.50	3.1-4.20	0			0	0			0												
6	5	3.60	3.2-4.30	0			0	0			0												
13	4	3.70	2.8-4.70	0			0	.3			.05												
11	4	3.90	3.2-5.00	0			0	0			.04												
14	2	4.10	1.8-5.40	0			0	0			0												
15	5	4.20	3.6-5.10	0			0	0			0												
15	5	4.20	3.6-5.40	0			0	0			0												
15	6	4.60	3.6-6.30	0			0	0			0												
8	7	4.80	3.7-6.00	0			0	0			0												
15	5	4.80	4.2-6.40	0			0	0			4.0												
9	5	5.20	4.3-6.60	0			0	0			.04												
6	5	5.20	4.4-6.00	0			0	0			.4												
5	5	5.40	4.2-6.40	0			0	0			.03												
12	2	5.50	4.3-6.90	0			0	0			.1												
11	2	5.60	4.8-6.80	0			0	0			16.0												
14	6	5.90	4.4-7.40	0			0	5.0			13.0												
7	6	6.40	6.4-8.80	0			0	0			5.0												
5	5	7.60	7.2-8.30	0			0	0			18.0												
10	7	8.00	9.5-11.00	0			0	2.0			5.0												
7	10.40	10.6-12.50		.13			0	2.0			15.0												
5	11.40	11.0-15.60	0	.7			0	10.0			30.0												
5	12.80	12.0-17.30	0	14.0			0	27.0			40.0												
13	4	13.90	13.6-15.90	0	19.0		0	37.0			38.0												
5	5	14.60	16.0-17.30	0	7.0		0	22.0			34.0												
15	5	16.50	15.7-18.80	0	27.0		0	27.0			27.0												
11	2	16.70	16.1-18.50	.03	58.0		0	67.0			67.0												
5	5	16.90	16.2-18.00	0	14.0		0	21.0			32.0												
14	2	17.10	17.2-20.00	0	17.0		0	21.0			32.0												
11	5	18.40	18.0-19.10	2.1	59.0		0	66.0			66.0												
11	5	18.60	17.2-19.90	.07	21.0		0	40.0			40.0												
12	2	19.10	17.6-20.20	3.1	42.0		0	40.0			40.0												
10	7	19.10	17.8-20.60	.3	18.0		0	27.0			27.0												
5	5	19.50	19.2-20.00	.7	18.0		0	27.0			27.0												
14	5	19.90	20.3-23.90	.05	16.0		0	27.0			27.0												
11	2	21.60	19.7-23.50	3.8	53.0		0	57.0			58.0												
5	5	21.70	19.7-23.50	2.7	29.0		0	47.0			47.0												



With these three considerations in mind, it may be concluded that the spores begin to germinate between  $4^{\circ}$  and  $5^{\circ}$  C., and that the maximum temperature for germination lies between  $31^{\circ}$  and  $34^{\circ}$ . If the optimum be arbitrarily chosen as that range of temperature over which germination with usual sporidial production will take place under these conditions in 24 hours, it is  $15^{\circ}$  to  $28^{\circ}$ . The minimum and optimum for sporidial production are the same as for germination, but the maximum is somewhat lower, ranging from  $29^{\circ}$  to  $30^{\circ}$ .

A few interesting observations may be noted here. In the broth solution the spores most commonly germinate into promycelia from which sporidia are cut off, in contrast with the germination on agar placed in the soil, where hyphae usually are produced in place of the sporidia. The effect of very high temperatures is evident in the type of germ tubes. At the lowest temperatures the tubes appear to be normal, whereas, at temperatures around  $29^{\circ}$  and  $30^{\circ}$  C., the sporidia sometimes seem not to be completely formed and the tubes tend to be thin and transparent. At temperatures around  $32^{\circ}$  the tubes are barely visible and never grow to the usual length.

#### THE INFLUENCE OF MOISTURE ON SPORE GERMINATION

The object of this phase of the work was to determine the effect of the moisture content of the soil on the spores of *Ustilago avenae*. Various methods, whereby spores could be placed in the soil under conditions similar to those obtaining in nature and recovered for examination under the microscope, were considered. The following technique finally was adopted. About 30 cc. of 2.5 per cent water agar of known acidity ( $P_{H}6.4$  or  $+0.4$  Fuller's scale) was poured into open petri dishes and dried thoroughly at a temperature not above  $60^{\circ}$  C. From the dried agar, smooth pieces 1 cm. square were cut. To distribute the spores evenly over the pieces of agar, a surface of paper was covered as evenly as possible with the spores. The pieces of agar were then laid one by one upon this surface and pressed gently to bring the whole of the surface in contact with the spores. Pieces of filter paper then were clipped above and below the agar with a fine wire. The spores thus prepared were sown under the same conditions as the oat seeds, 1 inch below the surface of the soil.

The soil moistures were made up on the basis of the moisture-holding capacity, as in the experiments of Bartholomew and Jones (2), and the acidity determined by Truog's (21) method. To insure uniform moisture distribution in the soil, either water was added to the soil 24 hours previous to sowing, or moistened soil was mixed before using, except that the 80 per cent was made originally to 60 per cent and after seeding was brought to 80 per cent. Uniform temperatures were maintained by incubators. The germination of the spores on the agar layer could be counted under the microscope after the removal of the filter paper.

At this point the question will arise as to how closely the conditions to which the spores were subjected in these experiments approximate the actual conditions to which the spores are exposed when naturally distributed in the soil. The availability of water to the spores is probably not the same when the spores are in contact with the agar as when they are in contact with the soil. In order to determine whether the amount of water absorbed by the agar varies directly with the moisture content

of the soil the following experiment was performed. Layers of oven-dried agar were weighed, clipped between filter papers, buried for 24 hours in cans of soil, the moisture content of which was made up to definite percentages, and then removed and weighed. The quantity of distilled water absorbed by 1 gram of oven-dried agar in 24 hours at 22° to 23° C. was found to be 11.8 grams. This maximum quantity of water absorbed under these conditions may be considered the water-holding capacity of agar. In the same time and at the same temperature similar agar placed in samples of a soil containing, respectively, 80 per cent, 50 per cent, and 20 per cent of its moisture-holding capacity, absorbed, respectively, 55 per cent, 30 per cent, and 10 per cent of its water-holding capacity. This shows that agar absorbs water approximately in proportion to the amount in the soil and may be interpreted as indicating that the amount of water available to the fungus fluctuates with the moisture content of the soil. It would seem that, as far as the other factors in the soil are concerned, the conditions to which the spores were subjected very closely approximated those occurring in nature, and it will so be assumed in the remainder of this paper.

In the first moisture experiments, the soil temperature was maintained between 20° and 24° C., and germination was recorded between 24 and 27 hours after sowing. Table III gives a summary of the results of these experiments.

From Table III it will be seen that the production of sporidia, or the small hyphæ which replace the sporidia, seems to be associated with conditions favorable to good germination, and occurs less frequently at 80 per cent soil moisture.

TABLE III.—*Relation of soil moisture to the spore germination of Ustilago avenae incubated at 20° to 24° C. in 24 to 27 hours*

Age of spores (months).	30 per cent water-holding capacity.		60 per cent water-holding capacity.		80 per cent water-holding capacity.	
	Germination.	Type of germination.	Germination.	Type of germination.	Germination.	Type of germination.
9.....	<i>Per ct.</i> 21	Threads, buds, <sup>1</sup> sporidia.	<i>Per ct.</i> 21	Threads.....	<i>Per ct.</i> 4	Short tubes, long tubes, buds.
11.....	19	Threads, sporidia.	19	.....do.....	0	
2.....	35	Threads, buds; sporidia rare.	28	Threads rare; buds abundant; very many short tubes.	.6	Tubes just visible; one long with thread.
2.....	16	Sporidia abundant; threads, buds, long tubes.	11	Long tubes, threads, buds.	5	Threads.
5.....	16	Very long tubes, threads; sporidia abundant.	10	Threads.....	5	Do.
5.....	18	Threads, sporidia very rare.	15	.....do.....	7	Few threads; long and short tubes; buds.
Average.	21	.....	17	.....	4	

<sup>1</sup> Threads are the infection hyphæ which may replace sporidia. Buds, a very early state of infection hyphæ or sporidia.

It was next desired to ascertain whether the same general relation of spore germination to varying soil moistures obtains at other temperatures. The low temperatures of  $10^{\circ}$  to  $13^{\circ}$  C., and the high temperatures of  $30^{\circ}$  to  $33^{\circ}$  were chosen for this study. The duration of the different experiments was determined by the length of time required for spore germination at those temperatures. Tables IV and V show an agreement with Table III, and the three tables make clear that at these different temperatures germination is highest at 30 per cent soil moisture, is usually less at 60, and falls off very markedly at 80.

TABLE IV.—Relation of soil moisture to the spore germination of *Ustilago avenae* incubated at temperatures of  $10^{\circ}$  to  $13^{\circ}$  C. in 48 hours

Age of spores (months).	30 per cent water-holding capacity.		60 per cent water-holding capacity		80 per cent water-holding capacity.	
	Germination.	Type of germination.	Germination.	Type of germination.	Germination	Type of germination.
9.....	<i>Per ct.</i> 24	Threads, some short tubes, long tubes, buds.	<i>Per ct.</i> 26	Threads very long; tubes, buds.	<i>Per ct.</i> 8	Threads, short and long tubes.
2.....	9	Sporidia, threads.	3	Long tubes.....	2	Short tubes.
5.....	9	Sporidia rare.....	5	Threads, long and short tubes.	5	Threads.
Average.	14	.....	11	.....	5	

TABLE V.—Relation of soil moisture to the spore germination of *Ustilago avenae* incubated at temperatures of  $30^{\circ}$  to  $33^{\circ}$  C. in 24 hours

Age of spores (months).	30 per cent water-holding capacity.		60 per cent water-holding capacity.		80 per cent water-holding capacity.	
	Germination.	Type of germination.	Germination.	Type of germination.	Germination.	Type of germination.
10.....	<i>Per ct.</i> 4	Short tubes, no sporidia, some tubes long, some buds.	<i>Per ct.</i> 3	No sporidia or threads, short tubes mostly just visible.	<i>Per ct.</i> 0.06	Tubes just visible.
2.....	12	Long tubes with few threads.	4	Tubes long and just visible.	.20	Do.
5.....	10	Long tubes, buds.	13	Threads, buds....	3.0	Threads, short and long tubes.
Average.	8	.....	7	.....	1.0	

These results raise the question of the reason for the decrease of spore germination at the higher moisture contents. An increase in water might bring about a change in other factors affecting germination. These factors may be markedly modified when the puddling point is reached. At 80 per cent soil moisture, puddling is easily brought about

by such mechanical agencies as jarring or rapid addition of water. It was observed that when such a point was reached in a few of the preliminary experiments, germination was very much lower than in the later experiments when puddling was avoided by very careful handling of the soil. It is obvious, then, that the condition of the soil containing 80 per cent of its moisture-holding capacity was not far removed from that of puddled soil. Among the several factors that may have been responsible for reduced germination in wet soil, that of absence of sufficient oxygen appears the most probable. For this reason experiments were carried on to determine whether the spores germinate in the absence of oxygen.

#### THE INFLUENCE OF OXYGEN ON SPORE GERMINATION

In the search for a method whereby oxygen could be excluded from a spore suspension which could be kept under observation, many devices

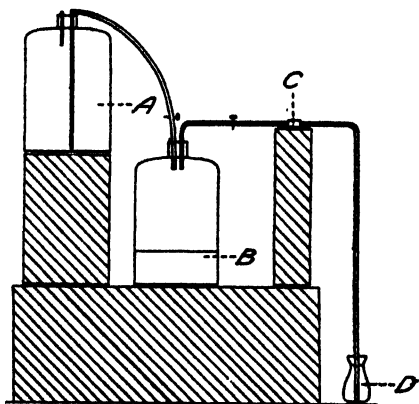


Fig. 3.—Apparatus for excluding oxygen from a spore suspension. A, reservoir of oxygen-free water; B, bottle containing pyrogallic acid; C, Engelmann cell; and D, bottle of water.

were tried before a satisfactory technique was finally adopted. The apparatus used is shown in Figure 3. Oxygen-free air was forced through an Engelmann cell (fig. 3, C) where spores were suspended in beef broth ( $P_H$  6.4), as in the germination experiments previously described. The beef broth had been freed from oxygen by heating at  $100^\circ C$ . and kept in an air-tight receptacle with a solution of pyrogallic acid. The oxygen was removed from the air in the air-tight bottle (fig. 3, B) by introducing a liter of alkaline pyrogallic acid and shaking it within the bottle for 20 minutes. Normal pressure was restored after shaking by siphoning boiled water from the reservoir (fig. 3, A) into the oxygen-free bottle. The tube from the reservoir to the bottle was then tightly clamped, the reservoir covered with a quarter of an inch of kerosene to exclude oxygen, and the latter raised to the position shown in the figure.

The Engelmann cell with its spore suspension was then attached to the outlet tube of the bottle, and a flow of water allowed to pass through the siphon into the bottle of oxygen-free air whose connection with the Engelmann cell was then opened. The oxygen-free air thus forced through the Engelmann cell was allowed to bubble through water in a small bottle placed at a much lower level (fig. 3, D).

By regulating the inflow of water from the reservoir, the oxygen-free air was forced rapidly through the cell for at least 40 minutes, and then more slowly for the remainder of the 24-hour period, except during the time when the air passing through the cell was collected for analysis in a Hempel pipette by the replacement of mercury. During each experiment four or five pipettes of gas were collected and the percentage of oxygen determined over phosphorus.<sup>3</sup> This analysis was made in order

<sup>3</sup> The writer is indebted to Dr. A. S. Loevenhart and the Department of Pharmacology, University of Wisconsin, for the use of the oxygen-analysis apparatus.

that the presence of oxygen entering the apparatus from any source might be detected.

With the apparatus in this final arrangement, three spore suspensions were thus subjected to an atmosphere free from oxygen as determined by the analysis of the samples of air passing over them. In no case did these spores germinate. Control mounts in Van Tieghem cells showed good germination. Before the apparatus was perfected and gas analysis made at the end of each germination, 15 trials had been made in which germination had occurred in only two instances when leaks in the apparatus, which admitted oxygen, were subsequently detected. The data confirm the results of the three final trials cited, and strengthen the evidence that *Ustilago avenae* spores will not germinate in the absence of oxygen. Some of these trials, however, have indicated that the spores will germinate in a low percentage of oxygen. In two experiments, germination of 5.9 per cent was found when the oxygen percentage was 5.85 and 1.95. The controls gave 28 and 35 per cent. In another trial in which germination was 27 per cent, and the control germinated 21 per cent, the gas analysis showed 0.5 per cent of oxygen.

#### DISCUSSION AND CONCLUSIONS

From the temperature experiments, it may be concluded that within the range from 9° to 28° C. there is no temperature effect upon the fungus which may prevent infection and establishment of the fungus within the host. According to Bartholomew and Jones (2) the range of temperature over which the highest percentages of smut occur is from 12° to 28°. This is nearly covered by the optimum range for germination of the fungus, 15° to 28°. Here also, according to Haberlandt (8, p. 43), is included the optimum for the most favorable and rapid germination of the host; namely, 25° C. At 9°, where the percentage of smut is either zero or very low, it takes about 10 days for the host to appear above ground and from four to five days for a good germination of the fungus and subsequent sporidial production. It would seem that the lack of smut was in no way due to an unfavorable influence of temperature upon the fungus alone. At 29° and below 6° C., however, the temperature seems to be unfavorable to the fungus. At the first and above, the germination is very much reduced and is abnormal, while sporidial production is rare. Here, it seems clear, a temperature effect may be the cause of the low percentage of smut. Below 6° it is possible that the host may develop before the germination of the fungus takes place, as the fungus requires about nine days to produce any considerable germination. These temperature conclusions are essentially in agreement with those of Ravn (20), Tubeuf (22), Eriksson (7), and Heald and Zundel (11).

As Bartholomew and Jones (2) found a low percentage of smut at the high soil moistures, and as at 80 per cent soil moisture there is a decidedly low fungal germination, with a slight falling off at 60 per cent as compared with 30 per cent, it seems safe to conclude that at the high content of soil moisture the decrease in spore germination may be a direct cause of the low percentage of smut. Analyzing this still further, we can perceive indications that this may be due to a factor involving oxygen supply. Were the oxygen completely eliminated at these high moisture contents, it could be stated conclusively that the absence of oxygen prevented the smut germination, for these experiments have demonstrated for this fungus the commonly assumed hypothesis that the spores



will not germinate in a liquid which is not exposed to oxygen. On the other hand, these experiments also indicate very strongly that with reduced oxygen supply germination still may take place. If it were true that in a soil of high moisture content some smut spores were in contact with oxygen and others were not, the reduction in germination would be clearly explained.

#### SUMMARY

(1) In order to determine the effect of temperature upon the germination of the spores of *Ustilago avenae*, spores were germinated in Van Tieghem cells or hollow-ground slides at a series of temperatures ranging from 4° to 35° C. in beef broth + 10 Fuller's scale (P<sub>H</sub> 6.1). The optimum for germination is arbitrarily defined as that range of temperature over which germination with usual sporidial production will take place under these conditions in 24 hours. The minimum temperature for germination was found to be between 4° and 5° C., the optimum from 15° to 28°, and the maximum between 31° and 34°.

(2) Under these conditions, the minimum and optimum for sporidial production are the same as for germination, but the maximum is somewhat lower, ranging from 29° to 30° C.

(3) The relation of moisture to spore germination was studied by placing the spores on layers of agar between filter papers in soil containing 30, 60, and 80 per cent, respectively, of its moisture-holding capacity. Germination was found to be highest at 30 per cent, to fall off slightly at 60 per cent, and very markedly at 80 per cent.

(4) *Ustilago avenae* spores failed to germinate in a suspension exposed to an oxygen-free atmosphere.

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# INFLUENCE OF TEMPERATURE ON THE SPORE GERMINATION OF *USTILAGO ZEAÆ*<sup>1</sup>

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Temperature has been regarded by investigators as important among the environmental conditions influencing the infection of corn by *Ustilago zeaæ* (Beckm.) Unger. Maire (5)<sup>2</sup> found that the spores of the corn smut fungus "germinate more quickly if the temperature is a little raised (20° to 25°)" and that the optimum temperature for sporidial and filamentous development is 20° to 25° C. Piemeisel (7), in considering the "phenomenon of infection and the optimum conditions for the propagation of the fungus," found the optimum temperature for the budding of sporidia to lie between 20° and 26° C., the maximum at about 40° and the thermal death point near 46°. He also found that incubation at 24° to 38° did not seem to influence the rate or amount of germination of the smut spores.

As the germination of the spores themselves would seem to be at least as significant as the budding of sporidia, and as the above investigators do not give detailed experiments on this stage of fungal activity, a study of the influence of temperature on the spore germination of *Ustilago zeaæ* was undertaken in order to throw additional light upon the relation of temperature to this early, significant stage in the life history of the fungus. The studies of which the results are presented in this paper have been carried on under the direction of Dr. L. R. Jones.

In searching for suitable media for spore germination it was attempted to duplicate natural soil conditions. Brefeld (2, p. 67-75) performed experiments which indicated that the germination of the *Ustilago zeaæ* spores takes place in the soil. The conception of soil, especially that containing manures, as a usual medium of germination of the spores also has been held even down to the present time. In attempting to find a soil medium like that obtaining in nature, the method of Thompson (8) was tested. When a tap-water suspension of spores was poured onto loam, no germination took place, whereas, when a suspension of spores in Pasteur's solution was introduced in the same way, germination followed. It seemed that the conditions for spore germination in such soil were not suitable, whereas they were satisfactory in the Pasteur liquid. In an attempt to find a soil on which the spores would germinate, seven modifications of loam and manure were employed, and the spores of *Tilletia tritici* (Bjerk.), Wint., were used as controls. On one pot which contained cow manure and loam, 1:2, there was considerable germination, and on another pot, one germinating spore was observed. Another test gave slight germination. In all these tests the bunt spores germinated abundantly, but, as a result of all the 27 attempts to germinate the spores of *Ustilago zeaæ* on soil, no satisfactory method was found.

<sup>1</sup> Accepted for publication Aug. 21, 1922.

The investigations, the results of which are presented in this paper, were conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, and the Department of Plant Pathology of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> Reference is by number (italic) to "Literature cited," p. 596-597.

An artificial medium was used, therefore, in the study of the effect of temperature on spore germination. Arthur and Stuart (1), Brefeld (2), Clinton (3), Hitchcock and Norton (4), Maire (5), and Norton (6) germinated the spores in many artificial media. Of these, Pasteur's solution was found most favorable for the following experiments.

The spores from a pustule formed in an anther were suspended in a tube of medium, mounted in Van Tieghem cells, and subjected to 13 different temperatures between 8° and 37° C. in thermo-regulated incubators. Duplicate mounts were placed at each temperature and the experiment performed 11 times. To determine the cardinal temperatures for germination, the percentage of germination was counted; the sporidial production, the number of cells in the promycelia, and the cell contents were observed; and the length and width of the germ tubes measured.

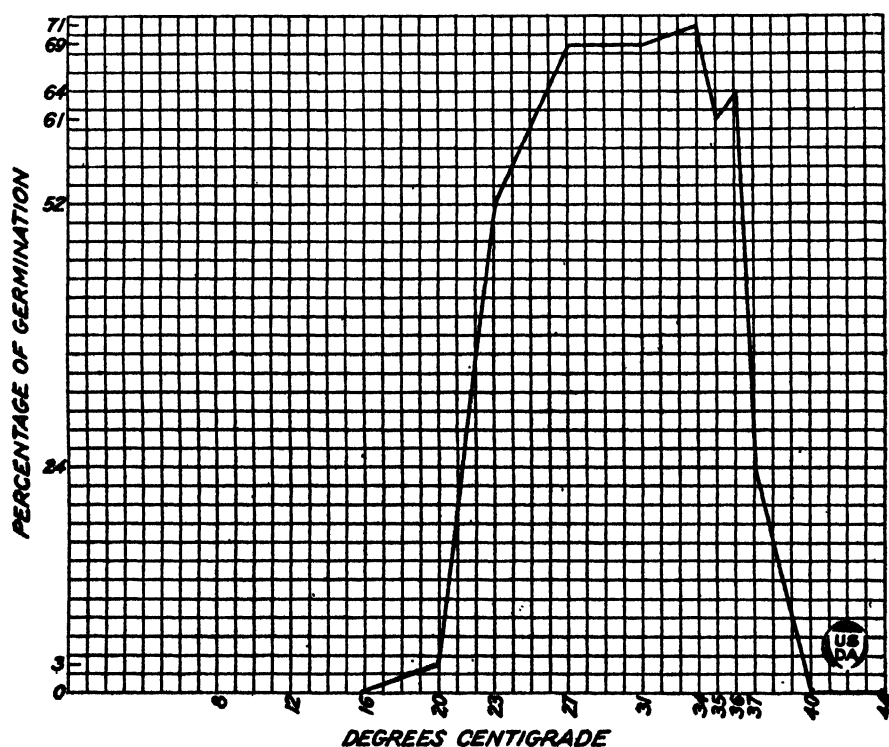


FIG. 1.—Graph showing percentages of germination of chlamydospores of *Ustilago zeae* at different temperatures.

The exact minimum for spore germination of *Ustilago zeae* was not determined. Four experiments showed germination at an average temperature ranging from 7.9° to 8.4° C., the germ tubes appearing on the 12th, 15th, 16th, and 21st days, respectively. The temperature range of the individual tests was greater than desired. That, together with the daily removal of the mounts from the incubators, may have given sufficient opportunity for eventual germination. The optimum temperature for germination is arbitrarily defined as that at which there is the highest percentage of germination within 24 hours together with a production of such germ tubes as behave normally, especially with respect to sporidial production. The highest percentage of germination within 24 hours occurred between 26.5° and 34.7° C. as is shown in Table I and figure 1, which give the average results of all experiments. As



determined by the number produced on first observation, the optimum temperature for sporidial production lies between  $26.5^{\circ}$  and  $34.7^{\circ}$  C. The maximum lies between  $36^{\circ}$  and  $38^{\circ}$ . Of 20 observations above  $34^{\circ}$  only 4 showed abundant production.

At the low temperatures, wherever germination took place sporidia also were produced. The number of cells in the basidia and the width of basidia were alike at the different temperatures and so could not be used as criteria in establishing the optimum temperatures. The lengths of basidia and condition of cellular contents, as shown in Table I, differed at  $36.1^{\circ}$  to  $37.8^{\circ}$  C. from those at other temperatures, indicating a deviation from normal at this high temperature. As below  $26.5^{\circ}$  the percentage of germination is decreased, and, as above  $34.7^{\circ}$ , the sporidial production is decreased and germ tubes are abnormal, the optimum temperature for spore germination may be stated as between about  $26^{\circ}$  and  $34^{\circ}$  C. As Table I shows that the maximum temperature rests between  $37.1^{\circ}$  and  $37.8^{\circ}$  and as six experiments show no germination at temperatures above  $38^{\circ}$  after two weeks, the maximum temperature for germination apparently lies between  $37^{\circ}$  and  $38^{\circ}$  C.

Attention should be called to the fact that this optimum temperature for germination is much higher for *Ustilago zeae* than for other smuts which have been studied in this laboratory. For example, *Ustilago avenae* germinates best between  $15^{\circ}$  and  $28^{\circ}$  C., and, according to Volkart (9), *Tilletia tritici* (Bjerk.) Wint. and *T. laevis* Kühn germinate best between  $16^{\circ}$  and  $18^{\circ}$  C.

#### SUMMARY AND CONCLUSIONS

(1) In 27 trials to germinate *Ustilago zeae* spores in soils containing different amounts of manure, there was considerable germination in only one, slight germination in another, and germination barely occurring in a third. The spores germinated readily in many artificial media, from among which Pasteur's solution was chosen for the experiments to determine the relation of temperature to spore germination.

(2) The optimum temperature for the germination of *Ustilago zeae* spores was found to lie between about  $26^{\circ}$  and  $34^{\circ}$  C., the maximum between  $36^{\circ}$  and  $38^{\circ}$ , and germination was observed to occur at the minimum temperature of  $8^{\circ}$ . The optimum for sporidial production lies also between about  $26^{\circ}$  and  $34^{\circ}$  C., the maximum between  $36^{\circ}$  and  $38^{\circ}$ , and sporidia are produced at the lowest temperatures at which germination was observed.

(3) The experiments showing that high temperature is most favorable for spore germination indicate that infection likewise is favored by hot weather whether the germination takes place in the soil or in water held upon the host plant.

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## SPORES IN THE UPPER AIR<sup>1</sup>

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### INTRODUCTION

As a part of the rust-epidemiology investigations which have been made since the spring of 1917 by the Office of Cereal Investigations of the United States Department of Agriculture in cooperation with several State experiment stations, attempts have been made to get as much information as possible on the dissemination of spores by air currents and to correlate the data with the spread of rust on host plants. The usual method in studying the distribution of spores of pathogenic fungi by wind has been to expose spore traps of various kinds near the surface of the earth. The possibility of spores being carried to higher altitudes by convection currents, whirlwinds, and other air movements has been recognized, and spore traps have been exposed on high buildings, on mountain tops, and other elevated points. However, it is desirable to know how many spores there are in suspension several thousand feet above the surface of the earth.

During the spring and summer of 1921<sup>3</sup> spore traps<sup>4</sup> were exposed on airplanes<sup>5</sup> in the hope of obtaining more information on the dissemination of aeciospores and urediniospores of *Puccinia graminis* and other rust fungi. The general results seem to be worth recording, although the problem of rust epidemiology was not solved.

### METHODS

Airplanes were used in preference to balloons, kites, or other similar devices, because long distances could be covered in a short time, spore traps could be exposed easily at different altitudes, and the direction of flight could be changed at will.

Ordinary microscope slides (3 inches by 1 inch), smeared lightly with vaseline on one side, were exposed in different ways. One method was

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<sup>2</sup> The authors wish to acknowledge the enthusiastic cooperation of the commanding officer at Kelly Field, San Antonio, Tex.; Maj. Jr. A. Rader, air officer for the Seventh Corps Area of the United States Army, at Fort Crook, Nebr.; Mr. W. I. Votaw, manager of the Aerial Mail Service Station at Omaha, Nebr.; and Mr. W. A. Kidder, of the Curtiss Flying Field (private), St. Paul, Minn. The authors are under deep obligation to these men, and to the pilots working under their direction, for their generosity and courtesy.

<sup>3</sup> The manuscript containing the results obtained in 1921 was submitted for publication before the 1922 results were available. On account of the delay in publication it has seemed desirable to include a few of the most important results obtained in 1922, although details are not given.

<sup>4</sup> The mechanical spore trap was devised by G. D. George, of the Office of Cereal Investigations and the University of Minnesota.

<sup>5</sup> The Air Service of the United States Army furnished the planes and pilots for the flights. The following men assisted in the work for the U. S. Department of Agriculture: E. B. Lambert, E. H. Ostrom, J. J. Christensen, D. L. Bailey, Wallace Butler, and S. P. Harter.

for the operator, seated in the passenger cockpit of the plane, to expose the slide by hand for the desired length of time and then return it to the slide box in which it had been carried. Another method consisted in placing inside of a bottle a microscope slide attached to a wooden paddle, the upper part of which extended through a hole in the cork of the bottle and served as a handle. After the slide had been exposed it was again placed in the bottle. This obviated the necessity of opening the slide box in order to remove or insert a slide, but was rather cumbersome, as it was necessary to carry as many bottles as there were slides to be exposed.

A third method was to expose the slides in a mechanical spore trap. Several types of trap were used, but one devised by Mr. George was the most satisfactory. This was made with six compartments, each containing a slide. It was fastened to the wing struts of the plane and was provided with a wire control which was operated by the observer in the cockpit. One pull on the wire opened the first compartment and exposed slide No. 1; a second pull closed this compartment and terminated the exposure of the slide. The other compartments were opened and closed in the same manner. (Pl. 2.) By this method slides could be exposed, one at a time, for the desired length of time, at various altitudes, and at widely separated points.

Flights were made in southern Texas during the latter part of April and during May, at St. Paul on June 25, and between Omaha, Nebr., and Cheyenne, Wyo., in July, 1921. The exposed slides were sent immediately in tight boxes to St. Paul, where spore counts were made.

## RESULTS

Some typical results of exposing slides are summarized in Tables I and II. It is evident that there are many fungous spores at altitudes as high as 11,000 feet above the surface of the earth. In examining the slides special attention was given to rust spores. However, a great many spores of many other kinds of fungi, as well as conidiophores, numerous pollen grains, glumes of grasses, and some small insects also were caught on the slides.

It was practically impossible to identify many of the spores definitely. It was certain, however, that there were many spores of *Puccinia*, *Alternaria*, *Helminthosporium*, *Cladosporium*, *Cephalothecium*, *Ustilago*, *Tilletia*, and *Scolecotrichum*. (Pl. 1.) Spores of *Alternaria* were by far the most numerous, and not infrequently they were found adhering in characteristic chains. Chains of *Cladosporium* spores also often were found on the slides. On some of the slides there were large clumps of smut spores, there being approximately 60 in one of the largest clumps. Urediniospores were caught more commonly than any of the other spore forms of rust fungi, although teliospores were not uncommon, and, in 1922, aeciospores also were caught. On one slide there was a spore of what appeared to be *Ustilago zeae* with a promycelium and about 20 sporidia still attached. It is possible that this spore may have germinated in the vaseline. This seems quite unlikely, however, as there was no indication whatever that any of the other spores had germinated, and it seemed more probable, therefore, that the spore with its promycelium and attached sporidia was carried up into the air after it had germinated.

TABLE I.—Results of exposing slides on airplanes

Slide No.	Location.	Date.	Altitude (above ground).	Length of exposure.	Spores and pollen grains on slides.						Total.
					Stem rust, uredinio-spores.	Leaf rust, <sup>1</sup> uredinio-spores.	Alternaria spores.	Helminthosporium spores.	Miscellaneous spores.	Pollen grains.	
			<i>Fest.</i>	<i>Minutes.</i>							
1	San Antonio, Tex.....	Apr. 27	1,000	1	.....	84	1	.....	28	34	
2	do.....	do.....	3,000	5	.....	84	5	.....	28	44	
3	do.....	do.....	3,000	5	.....	13	.....	8	47	68	
4	do.....	Apr. 28	4,000	2	1	29	.....	6	29	65	
5	do.....	do.....	Control.	.....	.....	.....	.....	.....	.....	0	
6	Waco, Tex., to San Antonio, Tex.....	Apr. 29	500 to 1,500	30	355	1	1	3	21	381	
7	do.....	do.....	1,500 to 3,000	30	335	3	1	23	38	400	
8	do.....	do.....	1,500 to 3,000	25	104	.....	.....	9	50	163	
9	do.....	do.....	1,500 to 3,000	25	70	.....	.....	8	144	222	
10	do.....	Apr. 28	10,000 to 12,000	15	1	.....	.....	.....	.....	1	
11	do.....	do.....	12,000 to 14,000	15	.....	.....	.....	1	.....	1	
12	do.....	do.....	14,000 to 15,000	10	.....	.....	.....	.....	.....	0	
13	do.....	do.....	15,000 to 16,000	10	.....	.....	.....	.....	.....	0	
14	do.....	do.....	16,000 to 16,500	10	2	.....	.....	.....	.....	2	
15	San Antonio, Tex.....	May 2	1,000	20	366	5	2	17	60	450	
16	San Antonio, Tex., to Uvalde, Tex ..	May 23	Control.	.....	.....	.....	.....	.....	.....	0	
17	do.....	do.....	1,000	2	3	23	15	2	5	48	
18	do.....	do.....	1,000 to 2,000	10	28	33	13	14	5	93	
19	do.....	do.....	1,000 to 2,000	30	80	86	36	1	15	218	
20	do.....	do.....	2,000 to 2,500	20	16	32	1	1	25	75	
21	do.....	do.....	8,000 to 9,000	5	4	36	10	1	9	60	
22	do.....	do.....	9,000 to 10,000	5	.....	9	19	.....	5	33	
23	do.....	do.....	10,000 to 11,000	5	.....	4	3	12	1	20	
24	do.....	do.....	11,000 to 12,000	5	2	7	1	.....	.....	10	
25	San Antonio, Tex., to Cuero, Tex.....	do.....	Control.	10	.....	.....	.....	.....	.....	0	
26	do.....	do.....	1,000 to 2,000	1	4	12	3	7	.....	26	
27	do.....	do.....	3,000	10	40	30	12	15	15	112	
28	do.....	do.....	3,000	30	80	56	35	3	25	203	
29	do.....	do.....	3,000 to 6,000	10	34	35	3	10	.....	82	
30	Omaha, Nebr.....	July 13	2,000	25	33	14	2ab	12	11	15	
31	Kearney, Nebr., to Gothenburg, Nebr.....	do.....	6,000	25	4	3	34	3	14	12	
32	North Platte, Nebr.....	do.....	Ground.	Over night.	2	13	10	2	7	6	
33	North Platte, Nebr., to Ogallala, Nebr.....	July 14	2,500	25	19	36	23	2	9	22	
34	Sidney, Nebr., to Pine Bluff, Wyo.....	do.....	7,000	25	3	2	4	.....	3	12	
35	Pine Bluff, Wyo., to Cheyenne, Wyo.....	do.....	8,200	30	1	.....	5	.....	.....	6	
36	Potter, Nebr., to Sunol, Nebr.....	July 15	2,500	20	1	1	19	18	1	40	
37	Gothenburg, Nebr., to Elm Creek, Nebr.....	do.....	2,000	25	126	34	ab	38	ab	68	
38	Elm Creek, Nebr., to Wood River, Nebr.....	do.....	3,500	25	101	28	ab	92	ab	17	
39	Valparaiso, Nebr., to Omaha, Nebr.....	do.....	6,000	35	15	1	11	8	11	1	
40	Cheyenne, Wyo.....	July 19	6,500	5	32	17	11	4	11	12	
41	Pine Bluff, Wyo.....	do.....	8,000 to 9,500	15	48	25	11	4	11	18	

<sup>1</sup> *Puccinia triticina* and *P. coronata*.<sup>2</sup> Exposed by hand.<sup>3</sup> ab=Abundant (over 200).<sup>4</sup> n=Numerous (100 to 200).

TABLE I.—Results of exposing slides on airplanes—Continued

Slide No.	Location.	Date.	Altitude (above ground.)	Length of exposure.	Spores and pollen grains on slides.						
					Stem rust, uredinio-spores.	Leaf rust, uredinio-spores.	Alternaria spores.	Helminthosporium spores.	Miscellaneous spores.	Pollen grains.	Total.
			<i>Fest.</i>	<i>Minutes.</i>							
42	West of Pine Bluff, Wyo.....	July 19	10,000	5	44	20	<sup>4</sup> n	8	n	10	....
43	Grand Island, Nebr.....	do.....	2,000	10	<sup>3</sup> ab	n	ab	n	n	n	....
44	Big Springs, Nebr., to Sidney, Nebr..	July 22	3,500	25	198	27	ab	....	ab	24	....
45	Sidney, Nebr., to Bushnell, Nebr.....	do.....	5,000	30	57	20	n	4	n	2	....
46	Lodgepole, Nebr., to Brule, Nebr.....	July 23	4,500 to 5,000	30	7	11	n	2	n	....	....
47	Brule, Nebr., to North Platte, Nebr..	do.....	5,000 to 7,500	35	5	6	57	1	50	25	144
48	North Platte, Nebr.....	do.....	Control.	.....	.....	.....	.....	.....	.....	.....	0
49	Curtiss Field, St. Paul, Minn.....	June 25	500	2	2	2	.....	.....	.....	.....	4
50	do.....	do.....	1,000	2	2	5	.....	.....	27	.....	34
51	do.....	do.....	1,500	2	3	8	.....	.....	1	.....	12
52	do.....	do.....	2,000	2	3	5	.....	.....	1	.....	9

<sup>1</sup> *Puccinia triticina* and *P. coronata*.<sup>2</sup> ab=Abundant (over 200).<sup>4</sup> n=Numerous (100 to 200).

TABLE II.—Results of exposing slides in a spore trap on the wing of an airplane and by hand from the cockpit for five minutes, at Fort Crook, Nebr., July 22, 1921

Altitude.	Manner of exposure.	Spores and pollen grains on slides					
		Stem rust, uredinio-spores.	Leaf rust, uredinio-spores.	Alternaria spores.	Helminthosporium spores.	Miscellaneous spores.	Pollen grains.
<i>Fest.</i>							
10,500	In spore trap.....	0	13	21	0	5	2
10,500	By hand.....	12	18	147	1	46	0
8,000	In spore trap.....	15	2	90	5	65	7
8,000	By hand.....	56	13	735	21	<sup>1</sup> ab	158
7,000	In spore trap.....	14	1	65	5	48	1
7,000	By hand.....	18	17	435	15	ab	36
6,000	In spore trap.....	7	1	60	1	11	10
6,000	By hand.....	6	15	ab	11	ab	50
3,500	In spore trap.....	14	0	84	2	22	2
3,500	By hand.....	14	0	ab	19	ab	11
2,000	In spore trap.....	4	6	95	3	41	3
2,000	By hand.....	32	35	ab	63	ab	17

<sup>1</sup> ab=Abundant (over 200).

TABLE III.—Results of exposing slides, coated with vaseline or glycerine jelly, simultaneously by hand for 3 minutes on June 14, and vaseline-coated slides by hand for 10 minutes on July 5, from airplanes in the vicinity of a 40-acre tract containing large numbers of escaped common barberry bushes, near Waukegan, Ill.

Slide No.	Date.	Altitude above ground.	Location.	Number of spores of <i>Puccinia graminis</i> on slides.			
				Aeciospores.		Urediniospores.	
				Vaseline.	Glycerine jelly.	Vaseline.	Glycerine jelly.
	1922.	Feet.					
1	June 14	100	Over bushes.....	4	6	0	0
3	do.....	1,000	do.....	5	15	2	1
4	do.....	2,000	do.....	6	6	1	2
5	do.....	5,000	do.....	1	7	0	0
6	do.....	7,000	do.....	3	0	0	2
7	do.....	10,000	do.....	0	4	0	0
8	do.....	12,000	do.....	0	1	0	0
10	do.....	2,000	10 miles away.....	4	5	1	2
11	do.....	2,000	15 miles away.....	2	2	0	1
12	do.....	2,000	25 miles away.....	1	0	0	0
13	July 5	100	Over bushes.....	14	.....	5	.....
14	do.....	100	do.....	8	.....	2	.....
15	do.....	1,000	do.....	7	.....	4	.....
16	do.....	1,000	do.....	10	.....	0	.....
17	do.....	2,000	do.....	11	.....	4	.....
18	do.....	2,000	do.....	9	.....	3	.....
19	do.....	6,000	do.....	4	.....	3	.....
20	do.....	6,000	do.....	7	.....	3	.....
21	do.....	2,000	Over Fort Sheridan.....	0	.....	2	.....
22	do.....	2,000	do.....	1	.....	1	.....

On a single one of the slides, exposed for five minutes at an altitude of 10,500 feet (about 2 miles), near Fort Crook, Nebr., 224 spores were caught. On a similar slide exposed for the same length of time at an altitude of 8,000 feet, 827 spores of known identity were caught, and in addition there were about 200 spores of unidentified forms, making the total about 1,000.

The highest altitude at which slides were exposed was 16,500 feet, or more than 3 miles above the surface of the earth. This slide was exposed between Waco and San Antonio, Tex., on April 28, 1921, and two urediniospores of what appeared to be *Puccinia tritici* were caught. It would not be safe, however, to conclude that spores normally occur at this height, as only a few flights were made at such extreme elevations. Spores are very numerous up to about 11,000 feet, but at higher altitudes they apparently are relatively scarce.

The number of spores caught per slide varied according to the method and place of exposure. Fewer spores were caught in the mechanical spore trap than on the slides which were exposed by hand. This probably was due to the fact that the spore trap was attached to the struts just above the upper surface of the lower wing, and it is quite probable that the air currents were deflected in such a manner as to miss the slides to some extent. By referring to Table II it will be seen that more spores were caught on slides which were exposed by hand from the cockpit than on those in the spore trap on the lower wing. In future work it probably would be well, therefore, to expose the slides on the fuselage or under the lower wing.

The real question, however, is not whether a great many spores are in the upper air, but whether they still are viable when they reach the surface of the earth. The difficulty of determining this conclusively is quite apparent. Germination tests were made, but some of them were inconclusive. The spores were embedded in vaseline, and when they were transferred to water they apparently did not imbibe much of it on account of the presence of the vaseline on the exospore. However, *Alternaria* spores which had been caught at 3,000, 4,000, 8,000, and 10,500 feet, respectively, germinated quite readily.

None of the urediniospores of *Puccinia graminis* germinated in 1921, but many of the tests were made too long after the spores were caught. In 1922, many more tests were made, and it was found that urediniospores germinated readily when caught at altitudes up to 7,000 feet. No tests were made on spores which had been caught at higher elevations. In one test, 64 per cent of the urediniospores germinated on a slide which had been exposed at 2,000 feet. The altitude at which the spores were caught apparently had little or no effect upon their viability. It seems quite likely, therefore, that urediniospores might be carried for long distances in the air and still retain their power to germinate. Germination tests were made of the spores on 24 different slides, and an average of 11.2 per cent of the urediniospores germinated. Considering the fact that the spores often were partially embedded in vaseline or glycerine jelly, a surprisingly large percentage germinated. A few aeciospores which had been caught at an elevation of 1,000 feet also germinated. Many of the other kinds of spores also germinated very readily.

#### GENERAL DISCUSSION

The results of these preliminary experiments indicate that large numbers of spores and pollen grains are carried several thousand feet above the surface of the earth during the growing season. Probably they are carried long distances by the upper air currents, the direction and velocity of which are quite different from those near the surface. If the spores retain their viability, as some of them quite probably do, it is conceivable that a local epidemic might occur in one locality as a result of the blowing in of spores from an infection center in another distant locality. It would be particularly desirable to know more about the dissemination of the spores of those pathogens which often seem to be disseminated over wide areas in some almost miraculous manner, such for example, as *Phytophthora infestans* and various rust fungi.

Attempts were made in 1922 to get data regarding the spread of black stem rust. Slides were exposed on airplanes in Nebraska, Kansas, and Oklahoma, before stem rust had developed in those States but when it was already present in Texas. No spores were caught, however, until rust began to develop near the area in which flights were made. Flights were made from Fort Sill, Okla., toward Denison, Tex., when there was a considerable amount of rust near Denison but none in Oklahoma. It was quite clear that the number of spores in the air decreased rather rapidly as the distance from the rusted area increased. The distance to which spores may be carried undoubtedly depends on many factors, and no final conclusions can be drawn from a limited number of observations. Certainly aeciospores and urediniospores are carried up as high as 10,000 feet, and more, above the surface of the earth. Unless they are brought down by rain or some other agency,

they probably are blown long distances. Just how far they are blown and just how important they are in starting rust far from the place where they were produced can not be stated definitely. More observations will be made.

The airplane is a great aid in studying the distribution of spores of pathogenic fungi. It is likely to be very useful in epidemiology studies and it also may be useful in determining the value of establishing quarantine lines.

#### SUMMARY

(1) In the spring and summer of 1921 spore traps were exposed on airplanes at various altitudes and at several places in the Mississippi Valley.

(2) Many spores of several different genera of pathogenic fungi, conidiophores, pollen grains, glumes of grasses, and small insects were caught on the slides.

(3) Spores and pollen grains were relatively abundant at altitudes up to 11,000 feet. They were relatively scarce at higher altitudes, but two spores of what appeared to be *Puccinia triticina* were caught as high as 16,500 feet.

(4) *Alternaria* spores which were caught at altitudes of 10,500 feet and less were viable; urediniospores and aeciospores of *Puccinia graminis*, caught at elevations of 7,000 feet and 1,000 feet, respectively, also germinated.

(5) Airplanes probably will be useful in studying the dissemination of spores of many pathogenic fungi, and probably will aid in the solution of problems connected with the development of epidemics of plant diseases.



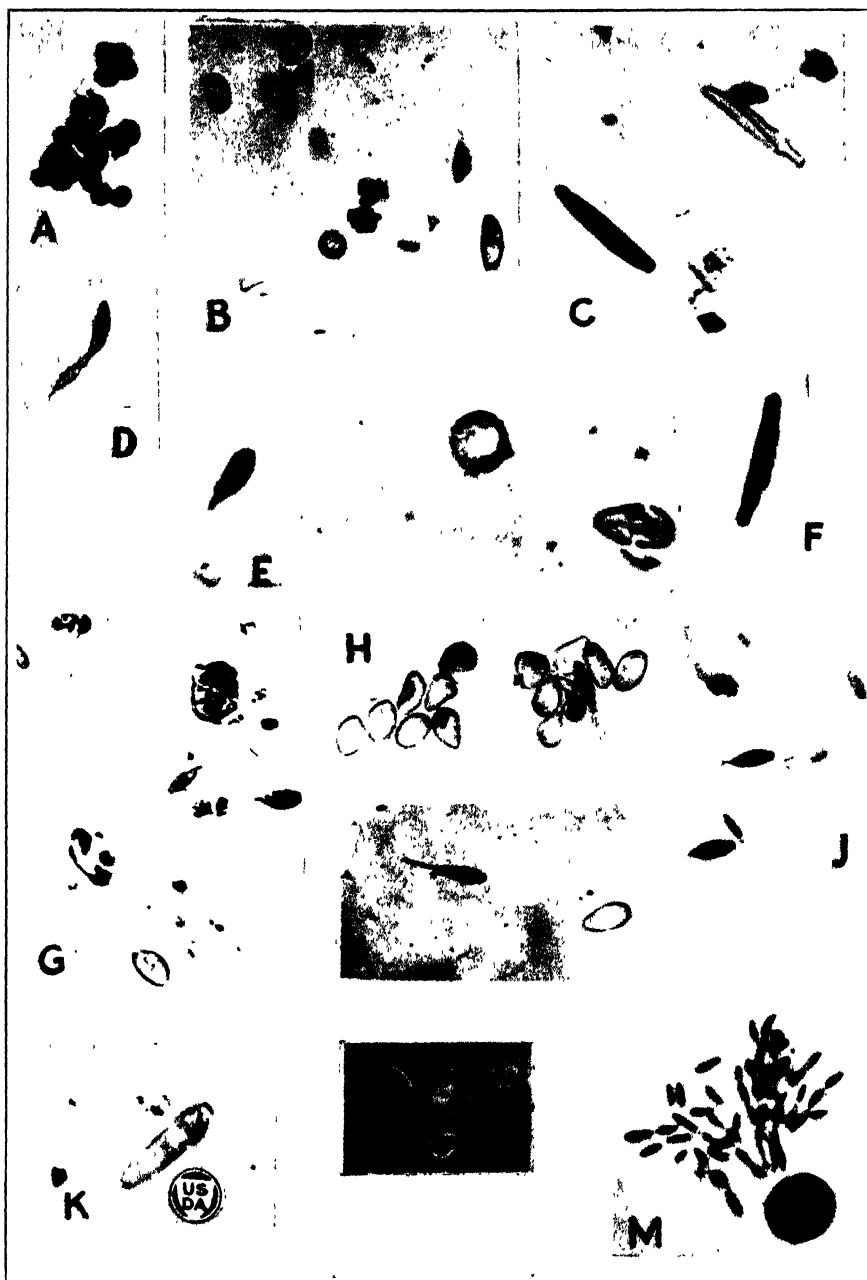
PLATE 1<sup>a</sup>

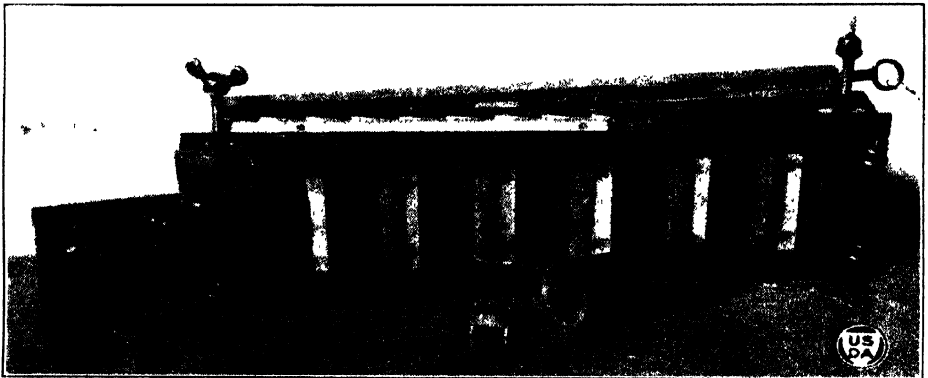
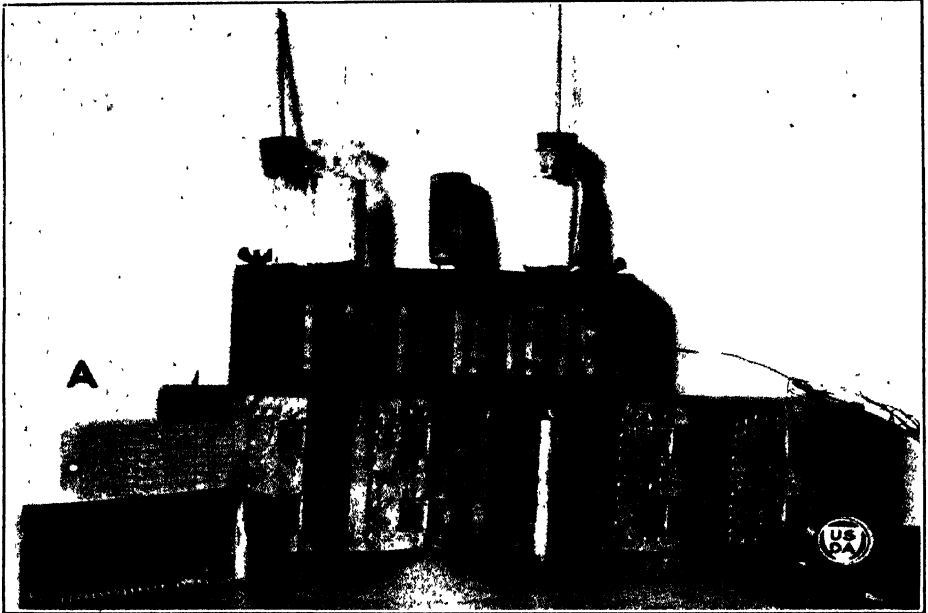
Photomicrographs of spores caught on slides at various altitudes.

- A.—Cluster of *Ustilago chlamydosporae* (3,000 feet, near Wahoo, Nebr., July 16).
- B.—Teliospore, *Tilletia tritici* spore, three rust urediniospores, and an *Alternaria* spore (1,500 feet, near Wahoo, Nebr., July 19).
- C.—*Helminthosporium* spore (10,000 feet, Pine Bluff, Wyo., July 19).
- D.—Chain of two *Alternaria* spores (1,000 feet, between North Platte and Gothenburg, Nebr., July 15).
- E.—*Alternaria* spore, leaf rust urediniospore, and a stem rust urediniospore (2,000 feet, near Fort Crook, Nebr., July 22).
- F.—*Helminthosporium* spore (1,500 feet, near Wahoo, Nebr., July 19).
- G.—Urediniospore of stem rust, two *Alternaria* spores, and a *Cladosporium* spore (10,500 feet, near Fort Crook, Nebr., July 22).
- H.—Cluster of rust urediniospores and one *Alternaria* spore (4,000 feet, between David City and Grand Island, Nebr., July 13).
- I.—*Alternaria* spore and stem rust urediniospore (10,500 feet, near Fort Crook, Nebr., July 22).
- J.—Two *Alternaria* spores and a *Cladosporium* spore (10,000 feet, near Pine Bluffs, Wyo., July 19).
- K.—*Scolecotrichum* (?) spore (3,000 feet, near Wahoo, Nebr., July 16).
- L.—*Fusarium* spore (10,500 feet, near Fort Crook, Nebr., July 22).
- M.—Budding spores or sporidia, exact identity unknown (3,000 feet, between Meade and David City, Nebr., July 13).

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<sup>a</sup> Taken with the following combinations: A, K, and M, with Zeiss microscope No. 47303, 18 oc. and 8 mm. objective; E, with Leitz microscope No. 161086, 7.5 oc. and 6 mm. objective; remaining figures with Zeiss microscope No. 47303, 10 oc. and 8 mm. objective.





**PLATE 2**

- A.—Types of spore traps used.**
- B.—Construction of George spore trap.**
- C.—View of George spore trap attached to plane.**



# STUDIES ON THE LIFE HISTORY OF STRIPE RUST, *PUCCINIA GLUMARUM* (SCHM.) ERIKSS. & HENN.<sup>1</sup>

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## INTRODUCTION

Extensive study has been made in European countries on the seasonal occurrence of the so-called yellow rust, caused by *Puccinia glumarum* (Schm.) Erikss. & Henn. It has been held by a few investigators in Europe that the disease may be transmitted from year to year by means of infected grain and grass seeds. Very little research has been carried on to determine if this is possible. The possibility of transmission of this rust through infected seed has an especial significance in the United States, due to its limited geographic distribution. It has been found that stripe rust in the United States is limited in its distribution to the States of the Pacific Coast and Rocky Mountain regions. If the disease can be carried on the seed, grain shipped from the Western States into the Mississippi Valley might become a means of spreading the rust into the Spring Wheat Belt.

It has been demonstrated by various workers that this rust can overwinter in the uredinal stage in central and northern Europe. It seemed important to know if this was true in the United States. Accordingly an investigation was undertaken, in cooperation with the Department of Plant Pathology of the Oregon Agricultural Experiment Station, in 1917, to work out the life history of this fungus under western conditions. The investigation was transferred, in 1920, to Moscow, Idaho, in cooperation with the Department of Plant Pathology of the Idaho Agricultural Experiment Station. The conclusions herein reported are the results of these investigations.

## LIFE HISTORY STUDIES

### OVERWINTERING UREDINIA

The aecial stage of this rust is unknown. Eriksson and Henning (3) were unable to find an aecial host for the rust, although they carried on rather extensive cultural experiments. It has not been possible so far to work upon this phase of the problem in connection with the present investigations. There have been no indications noted in the field which would suggest that there is an aecial host for the rust in this country.

It has been generally concluded by various authors in other countries that *Puccinia glumarum* is able to overwinter in the uredinal stage. Biffen (1)<sup>3</sup> states:

The uredospore stage seems sufficient to enable the fungus to tide itself over the winter, for it is possible to find pustules of rust on the foliage of self-sown wheat or some-

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<sup>2</sup> The author wishes to thank Prof. C. E. Owens for his hearty cooperation during the progress of this work, and also to acknowledge his indebtedness to Dr. H. B. Humphrey, Dr. A. G. Johnson, and Prof. H. P. Baras for helpful suggestions on the research and in the preparation of the manuscript.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 620.

times on the ordinary autumn-sown crop even in the depths of winter. The twisted leaves lying on the soil form a series of sheltered moist chambers on the inner surface of which the rust pustules are occasionally present in great numbers. These may develop with great rapidity in the early spring and at times as early as March the whole of the plant's foliage may be yellow with the rust. The winter's cold does not appear to injure these spores for they germinate readily when brought into the laboratory.

Hecke (4) concludes that *P. glumarum* can overwinter in Austria. He has observed the disease breaking out in the spring upon the old spots on the leaves which had been infected in the preceding autumn. In about two weeks this would be followed by a second generation appearing on the new growth of the plants. He concludes that the conditions necessary for an epidemic of yellow rust in any given year are a sufficient number of overwintering plants carrying mycelia and meteorological conditions favorable for its development in the spring.

Eriksson and Henning (3) conclude that *P. glumarum* may overwinter as mycelium in grain seedlings, and that this method is especially significant in years when there is a continuous covering of snow on the ground throughout the winter. They were doubtful if this means of overwintering is sufficient to carry the rust over from year to year. Henning (5) in a recent paper has reviewed the literature upon overwintering of this rust in Europe. He finds that the reports from various countries in central and northern Europe show that this rust can overwinter, at least when the host plants are protected, in the shape of uredinial mycelium. Such overwintering has been observed in Sweden by Eriksson, Henning, and Klebahn; in Denmark by Rostrup; in Austria by Hecke; in Bohemia by Baudys; in Russia by Treboux; and in Germany by Kirchner.

According to the author's observation there does not appear to be any doubt that the uredinial stage of stripe rust in either its urediniospore or mycelial condition can survive the winter in the mild climate of the coast region of the Pacific Northwest. When the winters are mild, it is possible to find fresh uredinia developing in any month from September to July in this region.

In the autumn of 1917 numerous varieties of wheat were sown at Corvallis, Oreg., in order to test them for resistance to stripe rust. A guard row of Chul wheat, which is very susceptible to stripe rust, was sown around the entire nursery in which these varieties were being grown. This entire border row was inoculated on November 21 and December 3 with urediniospores of *P. glumarum*. Infection spread continuously throughout the winter until by January 30 infection was quite general on all susceptible varieties of wheat adjacent to the inoculated plants. By March 1, all the susceptible varieties were heavily infected.

During the winter of 1917-18 a number of pots of the club wheat known as Dale (Dale Gloria), which is a susceptible variety, were grown in the greenhouse and inoculated with urediniospores of stripe rust until they were heavily infected. They were then placed out of doors in an exposed place for five weeks of the coldest weather during the winter. Snow fell three times during this period, and the plants, which were 6 to 8 inches tall, were killed down to half their length by the frost. In six days after they were again taken into the greenhouse they were covered with urediniospores on the portions of the leaves which had not been killed.

Observations also show that the fungus can overwinter readily on various wild grass hosts. In the fall of 1917 and also in 1918, observa-

tion stations were established near Corvallis, Oreg., with a number of wild grasses which were naturally infected with stripe rust. Plants of *Hordeum nodosum* L., *Bromus marginatus* Nees, and *Elymus glaucus* Buckl. were chosen for these observations. At no time during either winter was it impossible to collect viable urediniospores at any of these observation points. Viable urediniospores were collected upon *Elymus glaucus* in 1917-18 in every month from September, 1917, to July, 1918, inclusive.

There is some evidence also which indicates that the fungus may overwinter in the uredinial stage in the intermountain regions, between the Cascade and Rocky Mountain Ranges. Willis (11) reports that the rust may overwinter at Moscow, Idaho, on *Hordeum jubatum*. The author also obtained evidence from observations made in the spring of 1920, and again in 1921, that the uredinial stage of the fungus may survive the winter in northern Idaho. Plants of *Hordeum jubatum* and *Bromus marginatus*, which were heavily infected in the fall, developed fresh uredinia in both 1920 and 1921, soon after the snow melted in the spring. In the fall of 1921, various infected hosts in the grass nursery at Moscow, Idaho, and also other hosts located at various places near Moscow, were marked and were examined as soon as the snow melted in the spring. Snow covered these plants continuously from the last week in December until the middle of March. On April 3, 1922, several uredinial sori were found on an old leaf of a seedling of *Hordeum nodosum* growing beside a mature plant of the same species which was heavily infected in the fall. None of these sori had broken through the epidermis of the leaf. The spores were perfectly normal although immature. The appearance of the leaf seemed to indicate that infection had taken place in the fall and after remaining dormant through the winter had started to develop thus early in the spring. By April 15 a number of seedlings of this grass were found infected and producing urediniospores in abundance.

#### OVERSUMMERING UREDINIA

Observations made during the last five years indicate that along the Pacific coast of Washington and Oregon it is much more difficult for the rust to survive the dry summer months of July and August than to pass through the winter. The summers of 1917 and 1918 were exceedingly dry in the Willamette Valley of Oregon where observations were made during those two years. In 1917 there was no rainfall from June 8 until September 10, and in 1918 from June 5 until September 12. Needless to say, practically all of the wild grasses were completely dried up and very little rust infection of any kind was to be found by September.

Observations were made at Corvallis, Oreg., throughout the summer of 1917 upon *Elymus glaucus*, *Bromus marginatus*, and *Hordeum nodosum*, all of which hosts were heavily infected with stripe rust during the month of May. The *Elymus glaucus* plants were located on the banks of a small creek near the college farm. Urediniospores were produced in abundance upon these plants during May and the first half of June. Infection became gradually less until about July 20, after which no new urediniospores were produced until after the fall rains began. During this period, from July 20 to September 8, the *Elymus glaucus* plants made very little new growth, but a few leaves retained their green color. The old lesions or stripes showed plainly upon these half-dried leaves, but



no new spores were produced thereon. Soon after the fall rains began, however, uredinia began to appear at the ends of the old stripes where the mycelium had survived. The rust developed and spread rapidly until by October 15 an abundance of uredinial infection could be found upon the plants of this species in this locality. This same method of surviving the dry summer months was noted in the same place during the summer of 1918 (Pl. I), and upon *Hordeum nodosum*, *Bromus marginatus*, *Elymus glaucus*, *Elymus canadensis* L., and *Hordeum jubatum* in the rust nursery.

Waterhouse (10) has reported that in the hot dry summers of certain parts of Australia, *Puccinia triticea* Erikss. survives the dry period on volunteer wheat.

It seems evident, then, that one of the determining factors for an epidemic of stripe rust in any given year must be the weather conditions during the summer and fall of the previous year. If the precipitation during these months is insufficient to keep the grass hosts of *P. glumarum* and volunteer wheat more or less green in order to tide the fungus over this rather critical period, very little infection material will be present to start an epidemic upon winter wheat. It seems to the writer that the months from July to November in each year may well be considered the critical period for the fungus in regions where the winters are more or less mild. The meteorological conditions in the spring would need to be such as to favor the spread of the fungus which had survived the winter months. However, if fall-sown wheat becomes well infected in the fall, the early spring conditions are usually more or less favorable for subsequent spread. This, of course, would apply only to winter wheat. The weather conditions in the spring would undoubtedly be the determining factor in the infection of spring wheat.

#### RESISTANCE OF UREDINIOSPORES TO DRYING

It has been shown above that uredinial mycelium of stripe rust may survive the dry summer months in the tissues of the host. It seemed also important to learn the length of time in which urediniospores might remain viable under various conditions. Eriksson and Henning (3) found that the urediniospores of this rust were difficult to germinate unless they had been chilled to the freezing point or lower. The writer has had no difficulty in securing good germination of fresh urediniospores at any time. A number of trials were made to ascertain how long urediniospores would remain viable when kept at room temperature in herbarium packets. Table I gives the results of several of these trials.

TABLE I.—Percentage of initial and final germination of urediniospores of stripe rust, collected from different hosts, kept in paper packets at room temperature

Host.	Date collected.	Initial germination.	Date of final count.	Final germination.	Total storage.
		<i>Per cent.</i>		<i>Per cent.</i>	<i>Days.</i>
Wheat.....	Dec. 1, 1917	80	Jan. 12, 1918	1	43
Do.....	Nov. 27, 1917	90	Jan. 19, 1918	Trace.	56
<i>Agropyron dasystachyum</i> ..	Aug. 28, 1916	95	Oct. 24, 1916	1	58
<i>Elymus condensatus</i> .....	Sept. 5, 1916	85	.....do.....	Trace.	49

An experiment also was conducted to compare the relative resistance to desiccation of urediniospores of several rusts. *Puccinia graminis avenae* Erikss. & Henn. on oats, *Puccinia triticea* Erikss. on wheat, *Puccinia holceni* Erikss. on *Holcus lanatus* L. and *Puccinia glumarum tritici* Erikss. and Henn. on wheat, were chosen. Leaves of the several hosts, heavily infected with the uredinial stage of the rusts, were collected and several portions of the infected leaves were placed in each of a number of small glass vials which were then corked. Other infected leaves of each host were placed in three series of small paper herbarium packets.

The three series of specimens in the paper packets were exposed to different sets of conditions. The first series was placed on a shelf in the open laboratory; the second, out of doors on a window ledge on the north side of the laboratory, and the third on a shelf in a culture room. The temperature and moisture conditions in this culture room were a little more constant, and both temperature and relative humidity were a little higher, than in the open laboratory.

The glass vials containing infected leaves were divided into four groups. The first was placed on a shelf in the open laboratory, the second out of doors on a window ledge, the third in the culture room mentioned above, and the fourth in a small desiccator, the bottom of which was filled with water. This desiccator was kept in the culture room with the other collections. The vials in the desiccator were not corked. Table II gives the results of both these experiments.

TABLE II.—Resistance of urediniospores of various rusts to desiccation when stored in paper packets and in glass vials, under different sets of conditions

Kind of rust.	Where exposed.	How kept.	Date collected.	Date of final count.	Final germination.	Storage period
					Per cent.	Days.
<i>Puccinia graminis avenae</i> .	Laboratory .....	Packets...	Nov. 13, 1918	Feb. 4, 1919	0	82
Do .....	Window ledge.....	do.....	do.....	do.....	12	82
Do .....	Culture room.....	do.....	do.....	do.....	0	82
Do .....	Laboratory.....	Vials.....	do.....	do.....	0	82
Do .....	Window ledge.....	do.....	do.....	do.....	Trace.	82
Do .....	Culture room.....	do.....	do.....	do.....	0	82
Do .....	Desiccator.....	do.....	do.....	do.....	0	82
<i>Puccinia glumarum tritici</i> .	Laboratory.....	Packets...	do.....	do.....	10	82
Do .....	Window ledge.....	do.....	do.....	do.....	10	82
Do .....	Culture room.....	do.....	do.....	do.....	10	82
Do .....	Laboratory.....	Vials.....	do.....	do.....	10	82
Do .....	Window ledge.....	do.....	do.....	do.....	10	82
Do .....	Culture room.....	do.....	do.....	do.....	10	82
Do .....	Desiccator.....	do.....	Dec. 3, 1918	do.....	Trace.	63
<i>Puccinia triticea</i> .....	Laboratory.....	Packets...	Nov. 14, 1918	do.....	0	81
Do .....	Window ledge.....	do.....	do.....	do.....	5	81
Do .....	Culture room.....	do.....	do.....	do.....	0	81
Do .....	Laboratory.....	Vials.....	do.....	do.....	3	81
Do .....	Window ledge.....	do.....	do.....	do.....	0	81
Do .....	Culture room.....	do.....	do.....	do.....	0	81
Do .....	Desiccator.....	do.....	do.....	do.....	0	81
<i>Puccinia holceni</i> .....	Laboratory.....	Packets...	do.....	do.....	3	81
Do .....	Window ledge.....	do.....	do.....	do.....	5	81
Do .....	Culture room.....	do.....	do.....	do.....	Trace.	81
Do .....	Laboratory.....	Vials.....	do.....	do.....	Trace.	81
Do .....	Window ledge.....	do.....	do.....	do.....	Trace.	81
Do .....	Culture room.....	do.....	do.....	do.....	Trace.	81

<sup>1</sup> Trace of germination in 30 days

On January 8, 1919, urediniospores of *P. glumarum tritici* were taken from wheat leaves and placed on open slides and left under a bell jar in a culture room in the laboratory. The bell jar was raised slightly in

order to allow circulation of air. The spores were tested at the beginning and at intervals until no germination occurred. The germination at the beginning was found to be 76 per cent, at the end of 3 days it was 65 per cent, in 5 days 50 per cent, in 10 days 35 per cent, in 13 days 15 per cent, in 16 days 10 per cent, in 20 days 1 per cent, in 23 days only a trace, while in 25 days no more germination could be secured.

No extensive work has been done to ascertain how long urediniospores of *P. glumarum tritici* will remain viable in the field during the winter months. It is possible, as indicated above, to find viable urediniospores at almost any time during an open winter on the Pacific slope. It has not yet been possible, however, to learn if this is the case in the intermountain regions, where the winters are much more severe. Mr. A. F. Thiel states in a personal letter that he found, in connection with his studies of overwintering of stem rust in Montana, that urediniospores of *P. glumarum* were less resistant to the weather conditions than those of *P. graminis tritici*, *P. graminis avenae*, *P. graminis phleipratensis* or *P. triticina*.

#### TIME OF NATURAL INFECTION

Stripe rust is very commonly found upon young seedlings and much has been made of this fact by the advocates of the theory of seed transmission of the disease. Experiments were arranged during the winter of 1917-18 to ascertain how early in its development a seedling might become infected. Seedling plants of Chul wheat were inoculated at various stages in their development, beginning as soon as the plumule emerged and continuing with different plants at intervals until the first leaf was fully expanded. No infection was secured in any case where the inoculation was made before the primary leaf had begun to expand. Twenty seedlings grown from seeds germinated in Petri dishes lined with filter paper were used in each case. When the primary leaf was well expanded, these were transferred to soil in pots.

The details of the experiment are as follows:

No. 1.—Urediniospores placed upon the plumule just as it began to appear. No infection resulted.

No. 2.—When the plumule was one-half inch long, no infection resulted.

No. 3.—When the plumule was 1 inch long, no infection.

No. 4.—When the primary leaf was just beginning to unfold from the sheath, but not well opened, 10 per cent infection.

No. 5.—When the primary leaf was well opened, 100 per cent infection.

Abundant infection has been noted upon the primary leaf of seedlings of *Bromus marginatus*, *Hordeum nodosum*, *H. murinum*, *H. jubatum* and *Sitanion jubatum* which came up in the rust nursery at Corvallis, Oreg., around old infected plants of the same species. (Pl. II, A.)

Uredinia appeared upon young seedlings of Chul wheat 21 days after it was sown on June 10, 1917, alongside of some winter wheat which was heavily infected with stripe rust. (Pl. II, B.) Some of the same lot of seed was sown some distance from any known infection. No stripe rust appeared on the plants from this seed until two weeks after it had appeared upon the seedlings mentioned above. This indicates that infection may occur under field conditions as soon as the primary leaf unfolds. It is very evident from observations made during the winter and early spring, that seedlings infected in the autumn may be a very

common means of carrying the rust over winter, at least on the Pacific Coast.

#### PERIOD OF INCUBATION

The length of the incubation period of the uredinial stage of various rusts does not appear to be the same. Parker (8) found that the incubation period of *Puccinia graminis avenae* Erikss. and Henn. and of *Puccinia lolii avenae* McAlpine, on oats, was about the same. He states, however, that generally, though not always, the uredinia of the stem rust appeared first. He took notes on the appearance of flecks on the seventh to the ninth day, and notes on the formation of uredinia in 12 days. He calls attention to the effect of temperature and light upon uredinia formation. Low temperatures were found to lengthen the period. Durrell and Parker (2) state that the incubation period for crown rust upon seedlings of a susceptible variety of oats averaged 7.8 days. On older plants of the same variety the incubation period averaged 9.5 days. Biffen (1) found that the incubation period of uredinia of *P. glumarum* on wheat was about 10 days. Marryat (7) states that pale yellow areas appear 6 days after inoculation of susceptible varieties of wheat with urediniospores of *P. glumarum*. Pustules begin to appear on the eleventh and are all open by the thirteenth day.

The author's observations in the greenhouse in connection with inoculation with urediniospores of *P. glumarum tritici* on wheat indicate that with optimum conditions for infection, namely, a susceptible host, fairly high temperature after the first 48 hours, good light, and vigorously growing seedling plants, infection begins to appear on the eighth or ninth day and is evidenced by lighter-colored areas on the inoculated portions of the leaves. Spores appear in abundance on the twelfth or thirteenth day. As observed by Parker (8), low temperature and lack of sunlight may materially lengthen this period. It was observed in 1918 in connection with inoculations which were made in the field at Corvallis, Oreg., late in the fall and early winter, that the incubation period at that time of year might be as long as 30 days. It seems possible that uredinial hyphae, after penetrating the host and becoming established, may lie dormant for a much longer time under conditions unfavorable to the development of the host and then again continue growth and the production of spores when growth of the host is resumed.

#### HEAD AND KERNEL INFECTION

A number of writers have called attention to the fact that both uredinial and telial infection of a number of rusts may be found upon the caryopses of various grains and grasses. The author (6) has summarized the literature regarding the occurrence of this phenomena and has called attention to the fact that kernels of wheat infected with *P. graminis tritici* are rather commonly found in wheat from badly rusted plants. The percentage of rusted kernels was not large, however, in any sample examined.

The development of both uredinia and telia of *P. glumarum tritici* upon all parts of the head and kernel of certain varieties of wheat has been found to be very common during severe rust epidemics. In many cases of severe infection it is impossible to find a place on the entire plant from the surface of the ground to the tips of the awns that is not

covered with rust sori. Head infection, which usually indicates that the kernels are more or less infected, is common only in certain varieties of wheat. Chul (C. I. No. 2406), Dale (Dale Gloria, C. I. No. 4231), Talimka (C. I. No. 2495), Baart (Early Baart, C. I. No. 1697), Little Club (C. I. No. 4219), Jones Fife (C. I. No. 3452), and a number of others, are very commonly infected in the head even when the rest of the plant may not be heavily infected. Many times, the inside of the glumes and the kernels of plants of the above-named varieties may be heavily infected without the development of open sori on the outer surface of the glumes. Except in cases of very severe infection, only a few of the spikelets on each head are infected.

If infection develops on the heads soon after they emerge from the boot, sterility of a large number of spikelets may result. (Pl. III, A.) The kernels which develop in such heads usually are very badly shriveled. (Pl. III, B, and Pl. IV, A.) It is easily possible to pick out infected heads even though there be no open sori on the surface of the glumes and awns. Such heads are much lighter in color, being a yellowish green, while the rest of the plant is a normal green. Plate IV, A, shows a number of detached glumes from heads of Chul wheat collected at threshing time. The inside of these glumes shows an abundance of stripe-rust telia.

Kernel infection by stripe rust is much more common in certain varieties of wheat than the author (6) found to be the case in wheat infected with stem rust. As high as 60 per cent of the kernels of certain varieties were found to be infected when grown in a rust nursery at Corvallis, Oreg., where a heavy infection of stripe rust was secured by artificial inoculation. Over 35 per cent of infected kernels were found in several samples of wheat grown under field conditions. It seems to the author that the amount of head infection, and consequently the amount of kernel infection, depends largely upon the climatic conditions at heading time, as well as upon the variety of wheat.

#### EFFECT OF KERNEL INFECTION UPON GERMINATION

The author has reported (6) that kernel infection by *P. graminis tritici* does not affect the germinating power of wheat to any appreciable extent. This has not been found true of wheat kernels infected with *P. glumarum tritici*. Numerous germination tests were made with several varieties of wheat which were badly infected with this rust. Parallel series of unrusted kernels from the same samples of seed also were germinated at the same time. The germination of the rusted seed averaged about 50 per cent of that of the unrusted seed. In some cases the germination was less than 25 per cent of that of the unrusted seed. By reference to Plate IV, B, it will be noted that infected kernels are very much shrunken. In many cases a cross section of an infected kernel revealed dozens of sori under the pericarp over practically the entire surface of the kernel. In the case of wheat kernels infected with *P. graminis tritici* the sori usually were confined to the hilar region and were present only in limited numbers.

## SEED TRANSMISSION OF STRIPE-RUST INFECTION

## HISTORICAL DISCUSSION

The author (6) has reviewed the principal literature upon the subject of rust transmission by seed grain and discussed the three theories put forth by various writers to explain the means by which such transmission may take place. These are the mycoplasma theory, the dormant mycelium theory, and the seed-borne-spore theory. The results of experiments carried on by various workers along this line have also been tabulated in the author's previous work (6) on this question. This review will not be repeated here. In this paper carefully controlled field and greenhouse experiments with wheat infected with *P. graminis tritici* were also conducted. The results of these studies led to the conclusion that stem rust is not transmitted from one wheat crop to the next by means of infected seed grain. Waterhouse (9), more recently, has carried on similar experiments with wheat kernels infected with stem rust which also gave negative results.

Certain European investigators have secured positive results in experiments carried on to determine if *P. glumarum* can be carried from year to year on seed grain. Because of the economic importance of this phase of the question in the United States in connection with the spread of the rust to regions where it is now unknown, experiments were undertaken with stripe rust both in the field and under carefully controlled greenhouse conditions.

## EXPERIMENTAL DATA

A number of field observations have been made which at first seem to indicate very strongly that *P. glumarum* may be transmitted by means of seed. In the summer of 1917, among the increase plots of the Farm Crops Department at the Oregon Agricultural College, a plot of Jones (Winter) Fife was found to be heavily infected with stripe rust while none of the plots around it was infected to any extent. It was learned upon inquiry that the seed from which this plot was sown came from McMinnville, Oreg., and it was also learned that seed from the same lot sown at McMinnville produced plants that were not rusted. Upon close examination in the vicinity of the infected plots, numerous volunteer plants of Chul wheat were found which were heavily rusted. A plot of Chul wheat which was grown near this field the year before was known to have been infected with stripe rust. It seemed reasonable to suppose that the rust wintered over on these volunteer plants and infected the Jones Fife. No other very susceptible varieties were grown near this section of the field.

Another case in point which is not so easily explained was noted by Dr. A. G. Johnson, of the Office of Cereal Investigations, United States Department of Agriculture. Doctor Johnson found stripe rust on barley varieties in the nursery on the Belle Fourche Experiment Farm, at Newell, S. Dak., in August, 1917. These barleys were quite uniformly infected, and no other stripe rust was noted at Newell. The writer visited Newell in 1918, but was able to locate no stripe rust on or near the experiment station grounds. Some of the same seed from which these infected varieties had been grown was secured from Mr. John H. Martin, then of the Belle Fourche Farm, and sown in September, 1918. The plants from this seed were grown in the greenhouse at

Corvallis, Oreg., until they were headed but no rust appeared upon them. No rust infection could be found on this seed. Neither has the author been able at any time to locate any barley seed infected with *P. glumarum*. The work which is herein reported and the conclusions drawn relate to wheat only.

The investigations were along two lines: (1) Greenhouse experiments in which large numbers of rusted wheat kernels were sown under isolated conditions and the resulting plants watched for infection; (2) field experiments in which rusted wheat kernels were sown in the field and the resulting plants kept under observation to learn if infection occurred upon them sooner than upon plants grown from clean seed.

#### FIELD EXPERIMENTS

Rusted wheat seed has been sown for two years in comparison with clean seed and the resulting plants studied every few days for stripe-rust infection. The details and results of these experiments are given in tabular form, for each year separately.

In the autumn of 1917, rusted seed and chaff from rusted heads of several varieties of wheat were saved and sown in an isolated place on the college farm, near Corvallis, Oreg. The details of the experiment and the results secured are given in Table III.

These plots were examined carefully at frequent intervals in the fall and no stripe rust could be found on any of them at any time. In the spring the rust was slow to develop and did not spread rapidly, due to the extremely dry weather.

In 1919, a similar experiment was conducted under the same conditions. The details of the experiment and the results secured are given in Table IV.

As an additional precaution in securing seed which was known to be as free as possible from rust infection, all clean seed used was 1 year old.

The infection which appeared in the fall may have originated from some infected *Elymus glaucus* plants which were later found not far from these plots.

TABLE III.—*Development of rust on plants grown in the field at Corvallis, Oreg., in 1918, from rusted and unrusted seed sown on Oct. 10, 1917*

No.	Variety.	Condition of seed.	Number of rod rows.	Date rust first appeared.
1	Chul.....	Badly rusted seed and chaff.....	28	June 25, 1918
2	....do.....	Portions of infected heads.....	3	June 20, 1918
3	....do.....	Treated with modified hot-water treatment.	8	Do.
4	Talimka.....	Clean seed.....	2	June 22, 1918
5	Tigharia.....	Rusted kernels and chaff.....	3	June 25, 1918
6	Hansia Brooch.....	Rusted kernels.....	3	June 15, 1918
7	Popatia Nadiad.....	....do.....	2	May 31, 1918
8	Jones Winter Fife..	Clean seed.....	5	June 10, 1918
9	Chul.....	....do.....	5	Do.

TABLE IV.—*Development of rust on plants grown in the field at Corvallis, Oreg., in 1919, from rusted and unrusted seed sown on Oct. 20, 1918*

No.	Variety.	Condition of seed	Number of rod rows.	First rust appeared.
1	Salt Lake Club.....	Slight infection.....	10	None until spring.
2	Chul.....	Heavy infection.....	10	Do.
3	Dale (Dale Gloria)...	Clean seed.....	10	Do.
4	Baart (Early Baart)...	do.....	10	Do.
5	Talimka.....	do.....	10	Do.
6	Dale.....	Medium infection.....	10	Do.
7	Federation.....	Clean seed.....	10	Do.
8	Chul.....	Heavy infection.....	10	Jan. 29, 1919.
9	Baart.....	Slight infection.....	10	Dec. 15, 1918.
10	Hansia Brooch.....	Heavy infection.....	10	Feb. 11, 1919.
11	Popatia Nadiad.....	do.....	10	None until spring.

## GREENHOUSE EXPERIMENTS

In addition to the field experiments given above, greenhouse experiments have been carried on under controlled conditions. In order to grow a large number of wheat plants from seed infected with stripe rust, a portion of one of the greenhouses at the Oregon Agricultural College was equipped in the following manner: A partition was built to isolate completely one corner of the greenhouse; double doors were arranged in such a manner that anyone entering the isolated room could pass into a vestibule and close the first door before the second one was opened; the vestibule between the two doors always was sprayed with water before entering. A system of forced circulation of washed air was installed, as shown in figure 1.

The author (6) has reported upon this same system of air washing as it was used in connection with similar studies with wheat kernels infected with *P. graminis tritici*. Every precaution was taken to guard against any possible infection from outside. The room was made tight by using roofing cement to seal all cracks and openings in the sides and roof.

The first experiment was started on December 24, 1918. The seed used was for the most part from varieties grown in the rust nursery at Corvallis. It consisted of hand-picked kernels, all of which showed unmistakable evidence of stripe-rust infection. The varieties used were Hansia Brooch (C. I. No. 4690), Popatia Nadiad (C. I. No. 4696), Talimka (C. I. No. 2495), Baart (C. I. No. 1697), and Chul (C. I. No. 2406). In this, as in all other experiments of this series, temperature and humidity records were kept and it was found possible to maintain the temperature and relative humidity within the normal limits of plant growth. Flats filled with about 5 inches of sandy-loam soil were used for growing the plants. In all, 1,465 wheat plants were grown to maturity in this experiment, and no rust appeared on any of these plants at any time.

The second experiment was started on November 20, 1919. Wheat kernels were picked in the same manner as for the first experiment and the same methods were followed. Chul wheat (C. I. No. 2406) was used for the entire lot of plants in this experiment. The total number of plants grown was 2,470. No rust appeared at any time on any of the plants grown from this rust-infected seed and the experiment was discontinued when the wheat was mature.



In addition to the two experiments outlined above, 786 plants were grown from rusted seed in one of the rooms in the greenhouse without any special attempt to isolate the room to keep outside infection from the plants. These were grown to maturity during the winter months without the development of rust on any of them.

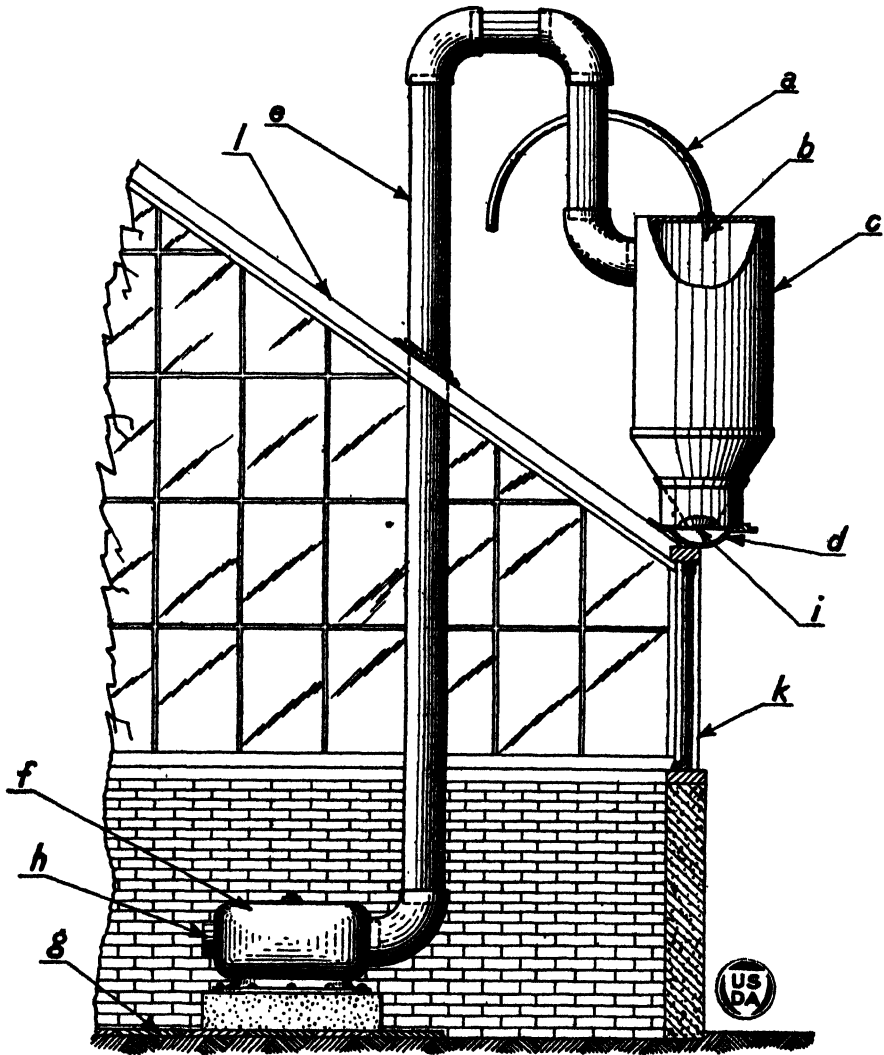


FIG. 1.—Diagram of air-washing apparatus for isolated room used for growing rust-infected seed: (a) Hose connection; (h) spray nozzle; (c) galvanized iron cylinder; (d) greenhouse gutter into which water from spray drained; (e) connection pipe from sprayer to blower; (f) electric blower; (g) floor of greenhouse; (h) mouth of blower where air entered the room; (i) air intake; (k) greenhouse wall; (l) greenhouse roof.

A number of writers have suggested the possibility of rust infection taking place from urediniospores on the surface of the seed or in the soil. To test this possibility, several flats were sown with wheat seed that had been covered with viable urediniospores of *P. glumarum tritici*. Chul wheat (C. I. No. 2406) was used for this experiment. About 300 plants were grown to maturity from seed thus treated. No rust infection appeared upon any of them at any time.

## SUMMARY

(1) Observations show that stripe rust, caused by *Puccinia glumarum* (Schm.) Erikss. and Henn., may overwinter on the Pacific coast, both as mycelium and as urediniospores, on wheat and wild grasses. It was possible to collect viable urediniospores during every month from September, 1917, to July, 1918, at Corvallis, Oreg.

(2) Although the experiments which have been carried on are not absolutely conclusive, there is good evidence that the mycelium of stripe rust may overwinter at Moscow, Idaho, on *Hordeum jubatum* and *Bromus marginatus*.

(3) It has been found that the mycelium of stripe rust passes the dry summer months on the Pacific coast as dormant mycelium in the leaves of wild grasses. It is suggested that the climatic conditions which may prevail during the summer and fall of a given year determine whether or not an epidemic of stripe rust may develop upon winter wheat. The quantity of oversummering uredinia would be an important consideration in producing an epidemic on fall-sown wheat.

(4) Germination tests with urediniospores of stripe rust show that when the leaves of the infected host are kept in herbarium packets at ordinary room temperature the spores may remain viable at least 58 days. Urediniospores on leaves of wheat kept in open vials in a desiccator gave a slight percentage of germination at the end of 63 days. Urediniospores taken from wheat leaves, placed on glass slides and kept in a protected place in the laboratory, gave a trace of germination in 23 days.

(5) Urediniospores of *P. glumarum tritici* did not prove to be so resistant to desiccation as urediniospores of *P. graminis avenae*, *P. triticea*, or *P. holceni*.

(6) It has been shown that infection by urediniospores of stripe rust can not take place before the primary leaf of the wheat seedling has begun to expand.

(7) The period of incubation for the uredinal stage of stripe rust has been found to be 12 to 13 days under conditions favorable for infection. Low temperature and lack of sunlight may materially lengthen this period.

(8) The uredinia and telia of stripe rust occur commonly upon kernels of certain varieties of wheat. As high as 60 per cent of the kernels of certain varieties grown in a rust nursery were infected. Over 35 per cent of infected kernels have been found in wheat grown under ordinary field conditions.

(9) The germination of seed infected with stripe rust was only 50 per cent of that of the uninfected seed from the same seed lots.

(10) Forty rows, each 1 rod long, of rust-infected seed of several varieties of wheat were sown on October 10, 1917. No stripe rust developed on the plants from this seed before the next spring. Plants from clean seed sown at the same time became infected the next spring at about the same date as the plants from the infected seed.

(11) More than 4,700 plants were grown from rust-infected wheat seed in a specially constructed room in the pathological greenhouse at the Oregon Agricultural College, and no rust appeared upon any of them at any time.

(12) No infection appeared upon plants grown from wheat seed which had been covered with viable urediniospores of stripe rust before sowing.

(13) The results of the experiments here reported indicated that stripe rust can easily overwinter in the uredinal stage on the Pacific coast and

in the intermountain regions of the West. They further indicate that *P. glumarum tritici* is not transmitted from one wheat crop to the next by means of infected seed wheat.

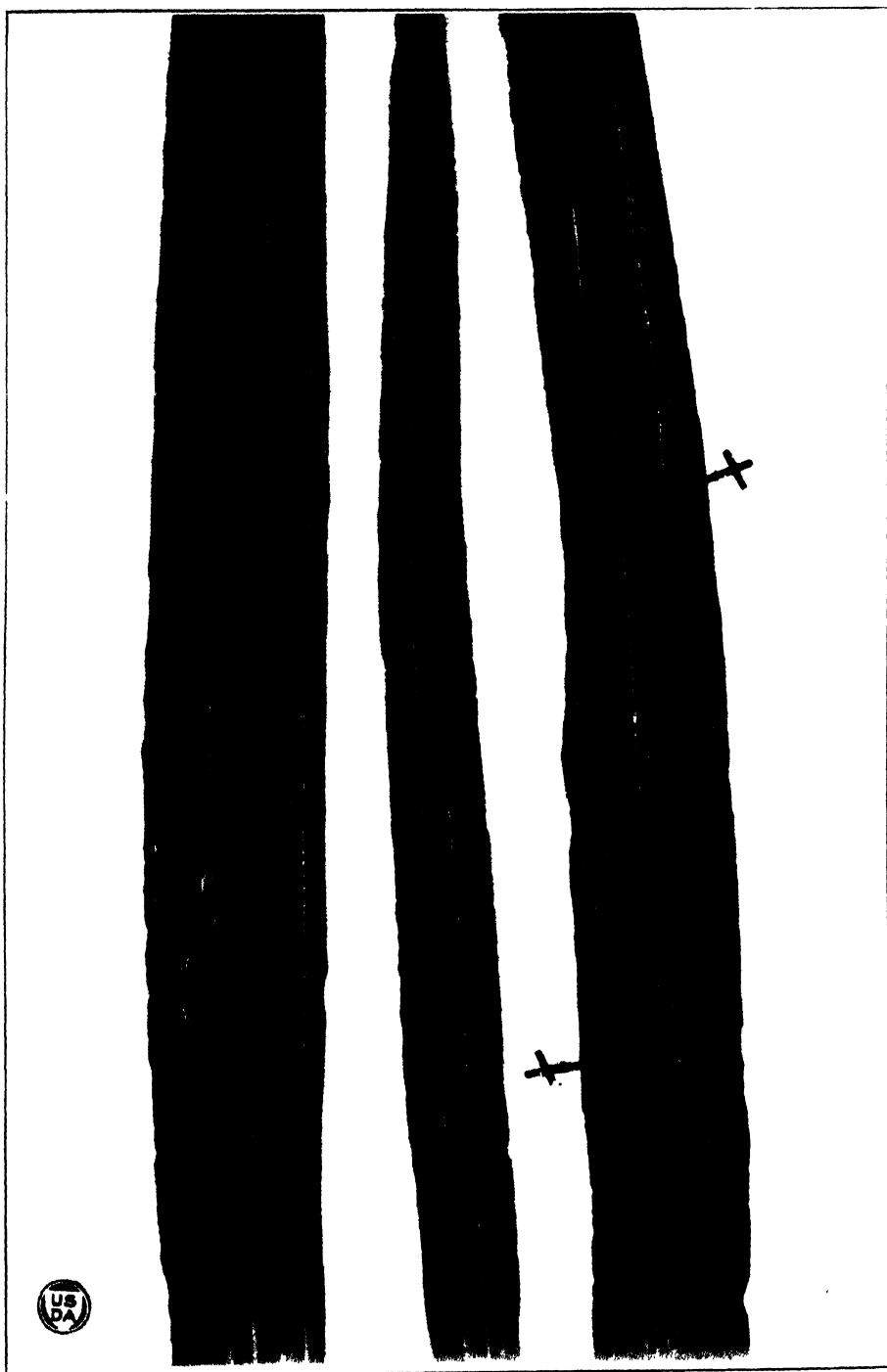
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# PLATE I

Leaves of *Elymus glaucus* infected with *Puccinia glumarum*. These leaves were collected on September 20. Note telia on the stripes. A few uredinia are beginning to break through at x. This illustrates the method of overwintering on the host in the Pacific Coast region.



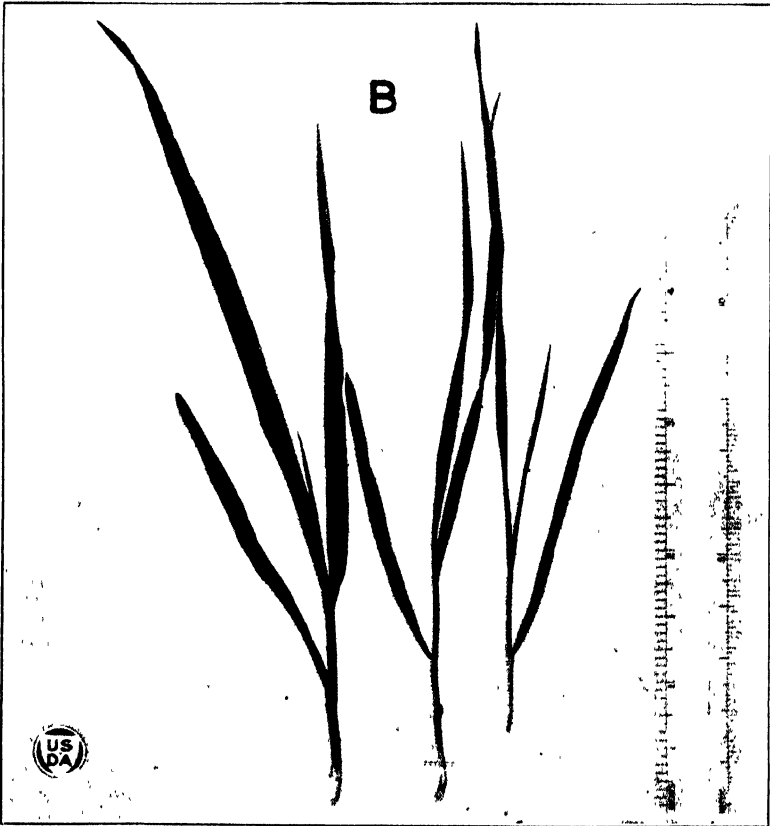
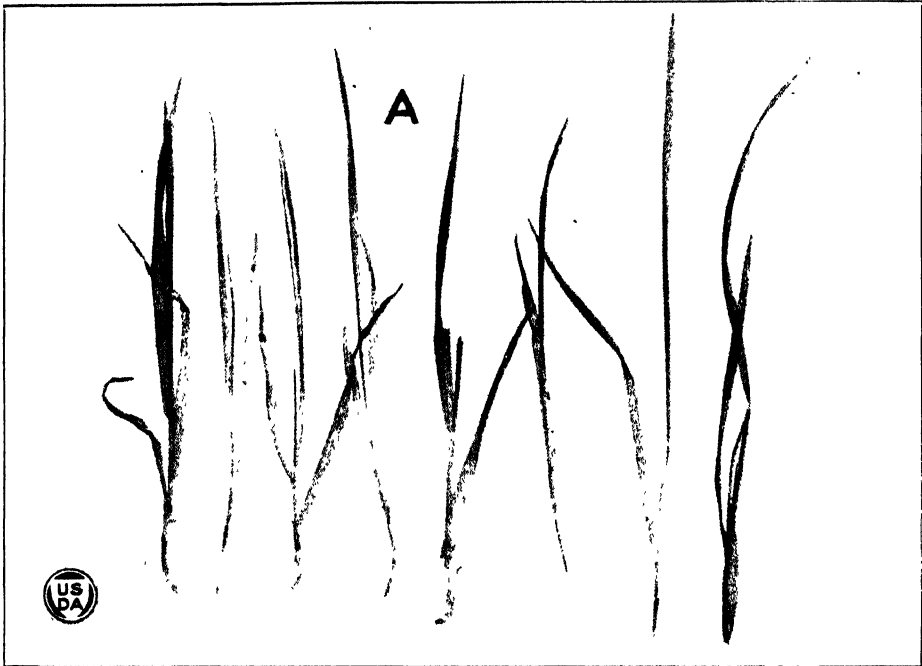


PLATE 2

A.—Uredinia of stripe rust on seedlings of *Hordeum nodosum*. These plants became infected from older plants around which they were growing.

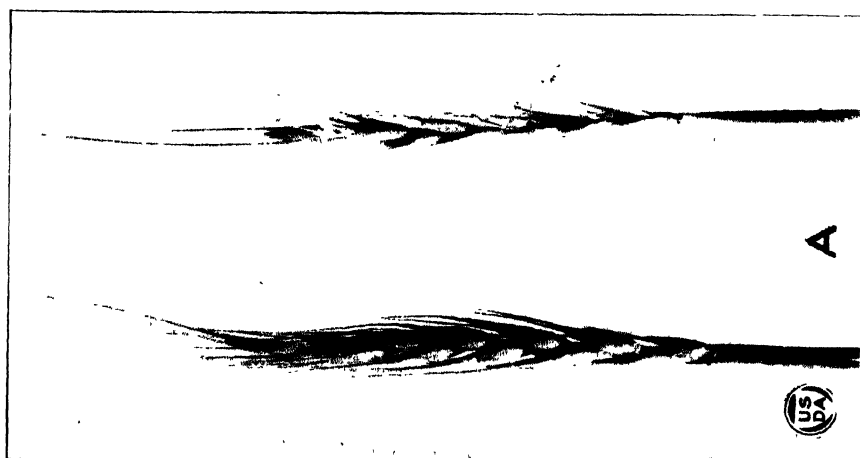
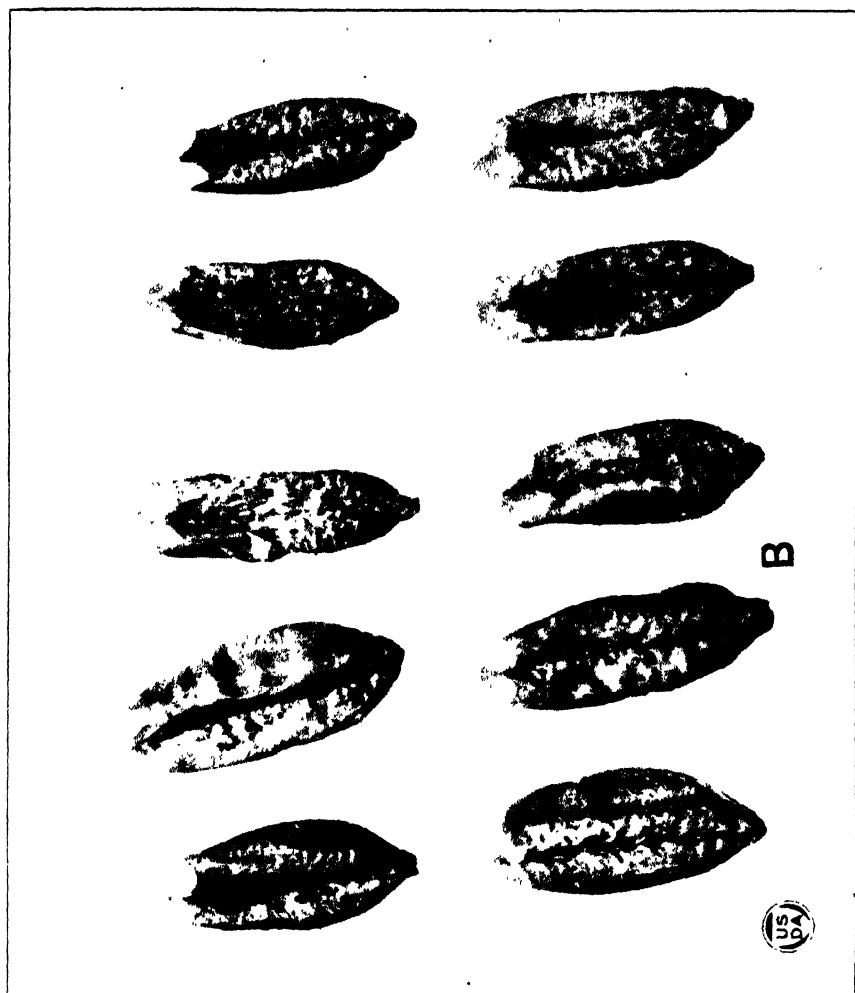
B.—Uredinia of stripe rust on seedlings of wheat which grew near older plants which were heavily infected. Seed from the same lot sown some distance from any infected plants produced seedlings free from the disease.



### PLATE 3

A.—Two heads of Chul wheat grown in the greenhouse. The one on the right was inoculated with urediniospores of *P. glumarum tritici*. The one on the left was not inoculated. Infection just as severe has been noted to occur under natural conditions in the field.

B.—More or less shrunk kernels of Dale (Dale Gloria) club wheat showing both telia and uredinia of *P. glumarum tritici* (much enlarged).



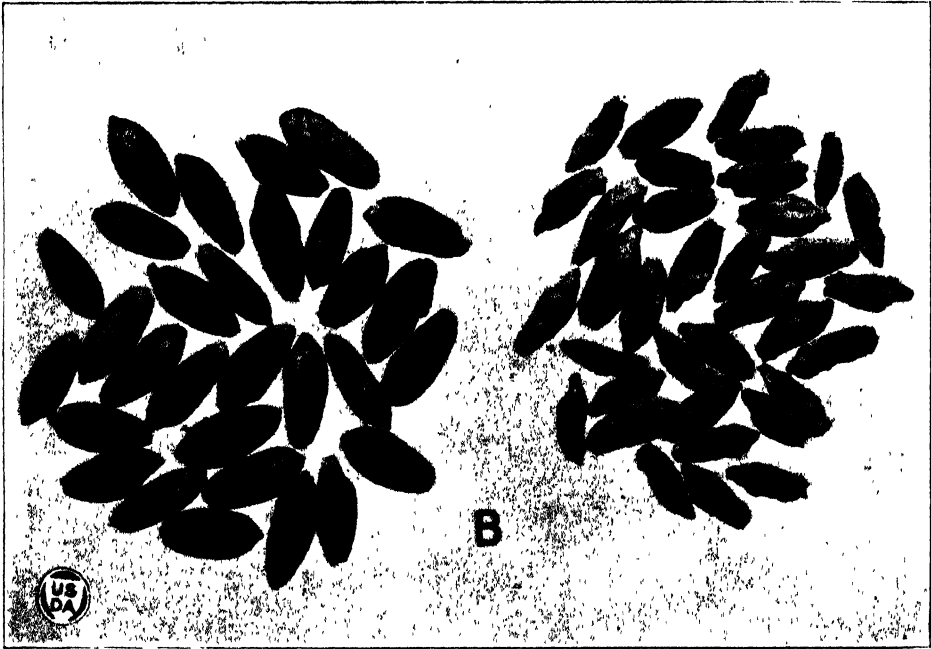
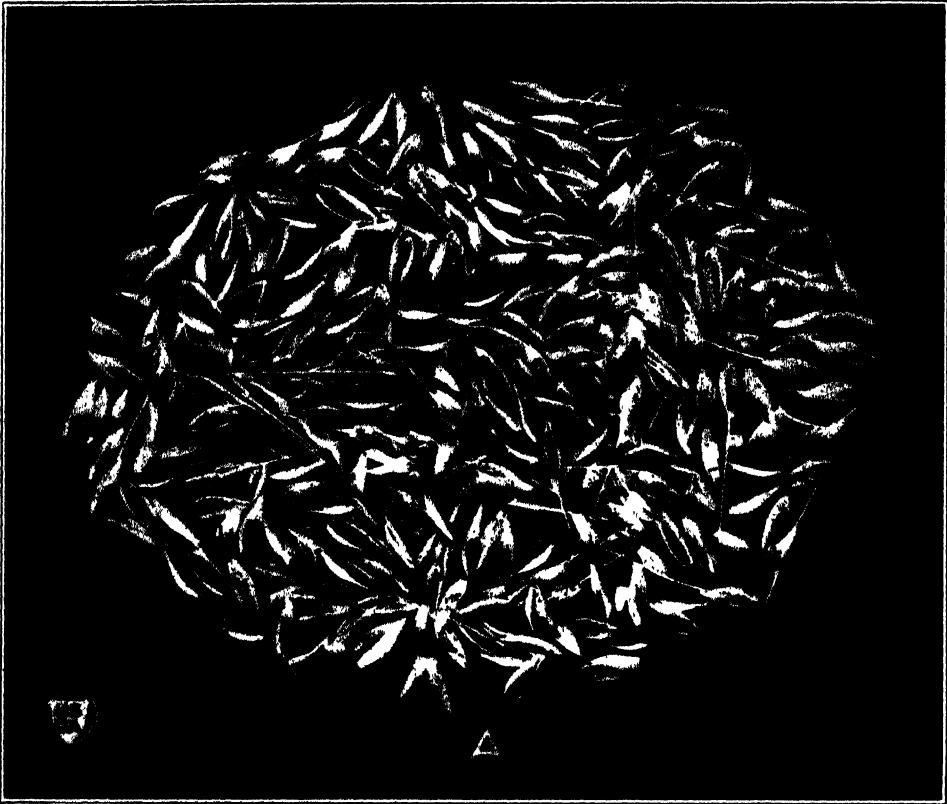


PLATE 4

A.—Telia and uredinia of *P. glumarum tritici* on glumes of Chul wheat. This chaff was secured from straw after threshing. (Enlarged.)

B.—Two lots of Chul wheat. Those at the right are infected with stripe rust. Those at the left are healthy kernels from the same sample. (Enlarged.)



# INFLUENCE OF SOME NITROGENOUS FERTILIZERS ON THE DEVELOPMENT OF CHLOROSIS IN RICE<sup>1</sup>

By L. G. WILLIS, *Chemist*, and J. O. CARRERO, *Assistant Chemist, Porto Rico Agricultural Experiment Station*

## DEFICIENCY OF IRON AS A CAUSE OF CHLOROSIS

The chlorosis affecting certain species of green plants when they are grown on calcareous soils has been extensively studied by Gile (6)<sup>2</sup> and Gile and Carrero (9), who carried on the greater part of their experimental work with rice and with pineapples. They offered strong evidence to support their opinion that such chlorosis is caused primarily by the precipitation of iron in the soil by calcium carbonate and the consequent inability of the plant to obtain a sufficient supply of iron for the development of chlorophyll.

Mazé, Ruot, and Lemoigne (16) have recorded that in some of their cultures even the small quantity of 0.2 per cent of calcium carbonate was sufficient to render certain species of plants chlorotic by depriving them of enough iron for the formation of chlorophyll.

## FACTORS GOVERNING THE AVAILABILITY OF IRON

### REACTION OF THE CULTURE MEDIUM

Gile and Carrero (8) further showed that in some solution cultures of neutral or alkaline reaction rice became chlorotic, and that such condition was attributable to precipitation of iron as phosphate and hydroxid. In their more acid solution, which had a reaction equivalent to a  $P_H$  of 3.1, these investigators noted an apparently higher degree of availability of all forms of iron used. In all these solutions nitrogen was supplied in the form of nitrates.

Working more recently with wheat in sand culture with nitrogen furnished in the form of calcium nitrate, McCall and Haag (18) have shown that solutions having  $P_H$  values ranging from 4.02 to 7.00 produced chlorotic plants, the color of which was not restored by the addition of ferric nitrate to the culture. The addition of very small quantities of sulphuric acid, however, resulted in marked improvement in the color of the plants, which became normal green at harvest time. These investigators were of the opinion that the indirect evidence supported the assumption that chlorosis in their less acid cultures was due to lack of available iron or to faulty metabolism resulting from immobility of iron in the plant.

### SOLUBLE PHOSPHATES

Factors in addition to the reaction of the culture medium are known to influence the development of chlorosis. Crone (2) advanced the hypothesis that soluble phosphates in solution cultures caused chlorosis of plants. Takeuchi (23), on the other hand, using wheat as a culture plant, showed that the chlorosis observed by Crone was probably due to the precipitation of iron as a phosphate in the culture medium. Later, Sidorine (22), working with corn, offered evidence to show that chlorosis,

<sup>1</sup> Accepted for publication July 11, 1922.

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 639-640.

due to lack of assimilable iron for the plant, may be induced either by an excess alkalinity or by the presence of large quantities of soluble phosphates in the nutrient. It is not improbable, however, that chlorosis that is due to precipitation of iron as phosphate could be obviated by proper adjustment of the reaction of the medium, though the degree of acidity necessary to accomplish this would be greater than it would be were the iron precipitated as hyroxid.

#### EXCRETION OF SOLVENTS FROM PLANT ROOTS

The reaction of the mass of a medium is not, however, necessarily the critical condition governing the availability of iron. Mazé, Ruot, and Lemoigne (16) attributed the resistance to chlorosis of some of their culture plants that were grown in alkaline media to the ability of the plants to excrete organic acids from their roots. These acids formed complex molecular compounds of iron that were soluble in the presence of calcium carbonate, and thus made available sufficient iron for the needs of the plant.

The solvent action of root excretion on insoluble matter which was in contact with roots was recognized by Takeuchi (23), who recommended the agitation of the precipitate in solution cultures to prevent chlorosis resulting from lack of iron. Mazé (15), too, stated that a marked influence was exerted on the plants in his solution when the roots reached the bottoms of the flasks or when the solutions were agitated and the precipitate settled on the plant roots. These observations indicate, as one significant difference between the reactions of plants grown in solution cultures and of those grown in soils, that, in the first instance, contact between the insoluble nutrients is not maintained and the solvent action of the root excretions is dissipated, and that, in the second instance, the influence of such material, being localized, exerts its maximum effect.

The excretion from plant roots of organic acids other than carbonic acid has, however, never been satisfactorily demonstrated. The results obtained by Kossowitsch (14) show that the elimination of carbon dioxide is apparently a normal function of the roots of many, if not of all, species of plants. It does not, therefore, seem probable that the variation in the susceptibility to chlorosis of different species of plants can be due under normal conditions to the excretion of a specific solvent.

It may be that variations in the quantities of carbon dioxide which are evolved from the roots of plants of different species determine the susceptibility of each plant to chlorosis when the plant is grown in less acid media; and as the source of this compound must be the carbon that is assimilated through the agency of chlorophyll, the activity of which in turn is dependent upon an adequate supply of iron, it is possible that in chlorotic plants the intensity of chlorosis depends, to a large extent, upon the balance existing between the potency of the agencies depressing the solubility of iron in the soil and the solvent power of the carbon dioxide that is eliminated from the roots.

#### EFFECT OF UNASSIMILATED RESIDUES OF FERTILIZER SALTS

It was shown by Kossowitsch (13) and by Mazé (15) that there might be a modification of the reaction of a culture as a result of plant growth and that, when ammonium sulphate was used as the source of nitrogen, the utilization of the basic radicle resulted in the release of the acidic radicle which, rejected by the plant at its roots, tended to produce an

acid condition of the medium. Similarly, when nitrates were used, the reaction of the medium was changed but in the opposite direction.

The effect of these residues on the insoluble nutrients in the soil had been demonstrated by Shulov (21), who found that when sulphate of ammonia was used as a source of nitrogen the insoluble phosphates in the soil were rendered more available to plants than when nitrogen was supplied as sodium nitrate.

Mazé, Ruot, and Lemoigne (17), too, suggested that the chlorosis, which developed in corn with which they experimented, was caused by the precipitation of iron by calcium carbonate that was liberated in the culture solution when the plant assimilated the acidic radicle of a calcium salt.

More recently, Jones and Shive (10) have shown that, in solution cultures of wheat plants, the availability of ferric phosphate, as indicated by the susceptibility of the plants to chlorosis, was governed by the degree of acidity of the medium which increased or decreased as a result of plant growth according to whether ammonium sulphate or calcium nitrate was used as a source of nitrogen.

It is probably true that in a solution culture in which there is no close contact between the plant roots and the precipitated material the reaction of the entire medium must be sufficiently acid to render the iron soluble in order to prevent chlorosis, and that the hydrogen-ion concentration at the threshold of chlorosis is the critical reaction governing the availability of the form of iron in that particular culture. In the denser media, however, in which the roots are in contact with insoluble material and in which circulation of the nutrient solution is retarded and sedimentation of precipitated matter is prevented, there may be, as a consequence of plant growth and the rejection by the plant of nonassimilable compounds, a very significant modification of the composition and reaction of the nutrient, the extent and intensity of which it is not easy to determine. Under such conditions it is evident that only that part of the culture which is in contact with the plant roots is the true medium in which the plant grows, and that the reaction of the unassimilated residue of the nutrient salts may be of more significance than is the reaction of the mass of the medium.

#### EFFICIENCY OF NITRATES AND AMMONIUM SALTS AS NUTRIENTS FOR RICE

It has long been known that rice (*Oryza sativa*) differs from the majority of other economic plants in that it does not seem adapted to the utilization of nitrate nitrogen as a fertilizer when it is grown under swamp conditions. Ammonium salts, on the other hand, have generally been found to serve as a suitable source of nitrogen, but Daikuhara and Imaseki (3) have noted that the difference between the efficiencies of the two forms of nitrogen disappears when rice is grown on soil that is not submerged.

Many attempts have been made to explain this characteristic preference of the rice plant for ammonium nitrogen. Nagaoka (19) suggested that three factors were involved in interfering with the utilization of the nitrates: (1) Loss of nitrogen by denitrification; (2) the formation of poisonous nitrites in the process of denitrification; and (3) the absence in the plant of sufficient sugar to transform the nitric nitrogen absorbed into protein.

Daikuhara and Imaseki (3) showed that the last theory was untenable when they proved by analysis that the sugar contents of paddy rice and of upland rice were approximately equal. Their results, however, indi-



cated that there was a great tendency to loss of nitrate nitrogen from leaching and denitrification in the submerged soil. They also observed that plants which were treated with nitrate became pale yellow in the early period of growth but that they recovered later. This was attributed to the physiological effect of poisonous nitrites which were formed by the reducing action of certain bacteria.

Kelley (11), too, found that rice made a weak yellow growth in pots when it was supplied with nitrates as a source of nitrogen, but that the plants usually assumed a deep green color as they neared maturity and apparently made normal growth thereafter. In one series of sand cultures to which sodium nitrate was added, however, it was observed that although repeated plantings were made, the seedlings in every instance stood for some days, turned yellow, and died. These results were considered to indicate that nitrates were unable to nourish the young seedlings properly, but Kelley also attributed injury causing the rice plants to turn yellow to the action of nitrites in the culture. Perciabosco and Rosso (20), however, showed that nitrites in culture solution were absorbed by rice without evidence of injury.

More recent work by Trelease and Paulino (25) shows that ammonium salts are more efficient sources of nitrogen for rice than are the nitrates. The results of Kellner (12) and of Espino (4), however, indicate that, while young rice plants require ammonium nitrogen for normal development, the older plants utilize the nitrates as well as they do ammonium salts. Gile and Carrero (8), on the other hand, have reported results obtained by the use of culture solutions in which nitrogen was supplied in the form of sodium and potassium nitrates; and while they did not claim that theirs was an optimum solution for rice, they stated that when the solution was sufficiently acid to insure the availability of enough iron, the rice made normal growth and was equal in size to exceptionally large field-grown plants. These investigators also observed that the yield of plants might be diminished by a deficiency of iron which was not great enough to affect the color of the leaves.

#### EXPERIMENTAL DATA

On account of the effect which the unassimilated residues of the different forms of nitrogen have on the reaction of plant culture media and also because of the relation existing between the reaction of the medium and the appearance of chlorosis, it seemed advisable to reconsider the question of the comparative efficiency of nitrates and ammonium salts for rice. It was therefore decided to make a study especially to determine the relationship between the two classes of compounds and the chlorosis due to the unavailability of iron for the plant.

For the earlier investigations the soil chosen was an acid red clay which, in a preliminary test, had been found to be very deficient in nitrogen and phosphorus. Plants that were grown on this soil were apparently immune to chlorosis regardless of the quantity of lime that was added to alter the reaction. In the response of plants on this soil to different forms of nitrates and ammonium salts no differences attributable to the form of nitrogen used were noted. The results, however, were rendered of doubtful value on account of an abnormality which was apparently the straight-head disease of rice that is described by Tisdale and Jenkins (24).

Further tests were made in which there was used an alkaline sandy soil from a river bank having the following composition:

*Analysis of moisture-free soil*

Constituents.	Per cent.
Material insoluble in HCl (sp. gr. 1.115).....	68.73
Ferric oxid ( $\text{Fe}_2\text{O}_3$ ).....	9.43
Aluminic oxid ( $\text{Al}_2\text{O}_3$ ).....	12.32
Manganese oxid ( $\text{MnO}$ ).....	.17
Lime ( $\text{CaO}$ ).....	2.02
Magnesia ( $\text{MgO}$ ).....	1.50
Potash ( $\text{K}_2\text{O}$ ).....	.20
Soda ( $\text{Na}_2\text{O}$ ).....	.38
Phosphorus pentoxid ( $\text{P}_2\text{O}_5$ ).....	.07
Sulphur trioxid ( $\text{SO}_3$ ).....	Trace.
Carbon dioxid ( $\text{CO}_2$ ).....	.37
Nitrogen (N).....	.034
Loss on ignition.....	5.47
Total.....	100.29

Preparatory to being used, the soil was put through a process to crush the lumps, and it was then passed through a screen (4 meshes to the linear inch). After being thoroughly mixed, 45 pounds of the air-dried material were then weighed into each of the required number of glazed earthenware pots having a 5-gallon capacity. No provision was made for drainage.

All treatments were added in solutions with sufficient water to saturate the soil, and the pots were placed on cars permitting of the carriage to a screened inclosure for exposure during fair weather and to a glass house at night and during rains.

Seed of the Wataribune variety was used for experimental plantings, a strain of which had been carefully selected for several generations for this purpose. Sufficient seed was planted to furnish about three times as many plants as were required, and the excess seedlings were removed so that the most thrifty plants would be well distributed in the pot. Plantings were made immediately after the treatments were added, and the soils were kept wet to the point of saturation until the plants were about 2 inches high. The soils were then flooded and kept covered to a depth of 1 or 2 inches until the plants were cut.

In a comparison made of several compounds to determine the best source of phosphorus for the soil, orthophosphoric acid ( $\text{H}_3\text{PO}_4$ ) was found to give satisfactory results. This form was, therefore, used in all the experimental work to obviate any interference by inert material which might mask the effect of other treatments.

#### EXPERIMENT I.—COMPARISON OF AMMONIUM SULPHATE AND SODIUM NITRATE TO DETERMINE THEIR INFLUENCE ON THE DEVELOPMENT OF CHLOROSIS IN RICE

Twenty-two pots of soil were given treatments of phosphoric acid and potassium sulphate to furnish each pot with 5 gm. of  $\text{P}_2\text{O}_5$  and 2 gm. of  $\text{K}_2\text{O}$ . The nitrogen compounds were added in sufficient quantity to supply 1 and 2 gm. of nitrogen to the pots which received the lighter and the heavier treatments, respectively.

Of the pots which received each treatment, one-half were sprayed by means of an atomizer every two days with a 1 per cent solution of ferrous sulphate, preliminary tests having shown that ferrous sulphate was as efficient for spraying purposes as was ferric chlorid or ferric citrate. Five plants were selected from the seedlings to remain in each pot. Table I gives the plan of the experiment and the significant results obtained from it.

TABLE I.—Chlorosis of rice on a calcareous soil as influenced by fertilization with sodium nitrate and ammonium sulphate

## SPRAYED WITH FERROUS SULPHATE

Pot No.	Nitrogen added as—		Analysis of solutions.										Weights of harvested plants.		Appearance of plants when cut.
	NaNO <sub>3</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	At planting.			At harvest.				Fresh.	Moisture-free.				
			Hydrogen-ion concentrations.	Parts per million of nitrogen present as—			Hydrogen-ion concentrations.	Parts per million of nitrogen present as—							
				NO <sub>3</sub>	NO <sub>2</sub>	NH <sub>3</sub>		NO <sub>3</sub>	NO <sub>2</sub>			NH <sub>3</sub>			
1.....	Gm. 2	Gm. ....	P <sub>H</sub> 7.6	213	0.6	0.7	P <sub>H</sub> 7.4	80	0.2	0.1	Gm. 133	24.9	Yellow spotted with dark green. Do.		
2.....	2	.....	7.8	288	.7	.7	8.1	80	.1	0	119	23.6			
Average.....			7.7	251	.7	.7	7.8	80	.2	.1	126	24.3	Dark green. Do.		
3.....			7.7	0	0	2.4	7.7	0	0	0	277	51.7			
4.....			7.6	0	0	1.8	7.6	.6	0	.1	242	47.3	Light green spotted with dark green. Do.		
Average.....			7.7	0	0	2.1	7.7	.3	0	.1	260	49.5			
5.....	1	.....	7.6	144	1.4	1.2	7.9	0	0	.1	110	23.8	Dark green.		
6.....	1	.....	7.6	163	1.4	.6	8.1	0	0	0	118	24.2			
Average.....			7.6	154	1.4	.9	8.0	0	0	.1	114	24.0	Dark green.		
7.....			7.8	0	0	.3	8.1	0	.1	.1	(a)	(a)			
8.....			7.7	.2	0	1.5	7.9	0	.2	.1	171	34.6			
Average.....			7.8	.1	0	.9	8.0	0	.2	.1	171	34.6			

NOT RAYED

9	2	..	7 6	250	1 5	0	8 4	90	0	0	85	14 1
10	2	..	7 9	325	1 1	0	8 3	140	0	0	120	21 2
Average	..	..	7 8	288	1 3	0	8 4	115	0	0	103	17 7
11	..	..	7 8	0	0	.6	8 0	0	0	.1	320	59 5
12	..	..	7 9	.2	0	.4	8 0	0	0	0	247	47 7
Average	..	..	7 9	1	0	.5	8 0	0	0	.1	284	53 6
13	1	..	7 7	150	1 3	0	7 9	0	0	0	128	25 3
14	1	..	7 8	125	1 5	0	7 9	0	0	.1	118	24 5
Average	..	..	7 8	138	1 4	0	7 9	0	0	.1	123	24 9
15	..	..	7 9	0	0	1 2	7 9	0	0	.1	161	32 4
16	..	..	7 9	.1	.0	1 8	8 0	0	0	.1	145	30 6
Average	..	..	7 9	.1	.3	1 5	8 0	0	0	.1	153	31 5
17	2	..	7 9	225	1 2	.2	8 3	140	.3	1 0	(b)	(b)
18	2	..	7 8	350	1 2	0	8 3	240	.2	1 2	(b)	(b)
Average	..	..	7 9	238	1 2	.1	8 3	190	.3	1 1	..	..
19	..	..	7 8	0	0	.5	8 3	.6	0	1 0	(b)	(b)
20	..	..	7 8	2	.1	.6	8 4	1 2	0	1 6	(b)	(b)
Average	..	..	7 8	.1	.1	.6	8 4	.9	0	1 3	..	..

<sup>a</sup>Lo:  
<sup>b</sup>No: planted.

The plants in this series of pots were cut while they were in the boot stage, it having been observed that those in soil cultures that had been rendered partly chlorotic by means of fertilizer treatment generally recovered their normal green color at about this period.

The results of this test show the influence on the development of chlorosis of the form of nitrogen used as a fertilizer and that the nitrate furnished was probably active in causing chlorosis, the effect being more marked on those pots receiving 2 gm. of nitrogen in that form than it was on those pots to which 1 gm. was applied. It would seem, therefore, that the calcareous soil was not the sole causative factor in similar cases of chlorosis, although this point has not been definitely established by the results obtained from the experiment.

That chlorosis was due to lack of sufficient iron for chlorophyll formation was evidenced by the development of a green color where the leaves were wet with the solution of ferrous sulphate. The nature of the leaf surface, however, was such that it was not possible to wet the entire area with the spray solution, and the plants therefore presented a mottled appearance. For this reason it is not certain whether the plants that were fertilized with sodium nitrate would have made a growth equal to that of the plants which received the ammonium sulphate had the spraying been more efficient as a remedy for chlorosis.

Observations made in this test do not show whether the plants which received sodium nitrate were chlorotic as a result of the form of nitrogen used or on account of the basic residue; whether the chlorosis, if caused by the basic residue, was due to the alkalinity of that residue or to the specific action of the sodium ion; nor whether there was a depression in plant growth additional to that caused by chlorosis.

A wide variation was observed in the size of the individual plants in the same pot and also in the tendency of plants of different sizes to recover from chlorosis. Apparently, absolute uniformity of cultural condition was not maintained in the experiment and the variations present governed the severity of the chlorosis.

Samples of the soil water for analysis were drawn from the mass of the soil by means of a pipette. The method of sampling was not ideal for the accurate determination of the composition of the nutrient solution, but it was chosen because it permitted the least disturbance of the plant roots. The determinations of hydrogen-ion concentration that were made by the colorimetric method of Clark and Lubs (1) are believed to be reasonably accurate, but the results showing the concentration of the different forms of nitrogen are only approximately correct.

The failure of the analysis to show the presence of appreciable quantities of ammonia is probably due to the absorption of that nutrient by the soil. Such removal of nitrogen from solution did not, it is thought, in any way interfere with the ability of the plant to absorb a sufficient amount for efficient utilization.

Evidence of the loss of some nitrogen through denitrification is shown by the decrease of nitrate nitrogen in the uncropped pots No. 33 and 35 during the period in which the plants were growing in the other pots. This loss, however, is thought to be of little significance in a comparison of the effects of the two forms of nitrogen.

The small quantity of nitrate nitrogen which accumulated in pots No. 34 and 36 as a result of the nitrification of ammonium sulphate may indicate that, as Fraps (5), Kelley (11), and Kellner (12), and others have shown, nitrification does not take place in submerged soils; or it

may indicate that denitrification processes were rapid enough to prevent the accumulation of considerable amounts of nitrate nitrogen. It is assumed, however, that in this experimental work the nitrification of ammonium sulphate was so greatly inhibited that the plants which were grown in pots treated with that compound derived the greater part of their nitrogen from the ammonium radicle. Nitrogen in the form of nitrite did not appear in significant quantities in any pot.

EXPERIMENT II.—A COMPARISON OF AMMONIUM SULPHATE AND AMMONIUM PHOSPHATE TO DETERMINE THEIR INFLUENCE ON THE DEVELOPMENT OF CHLOROSIS IN RICE

No direct method having been devised to eliminate the effect of the basic residues of the nitrate fertilizers in this soil, in order to test the effect of nitrate nitrogen on the development of chlorosis it was planned to determine whether or not resistance to chlorosis was characteristic of plants grown with ammonium compounds.

Thirty-four pots were prepared in the same manner as were those in experiment I. Potassium sulphate ( $K_2SO_4$ ) was applied in sufficient amounts to furnish 2 gm. of potash ( $K_2O$ ) to each pot, and phosphoric acid ( $H_3PO_4$ ) and ammonium sulphate [ $(NH_4)_2SO_4$ ] were used as sources of phosphorus and nitrogen in one-half of the number of pots, while monoammonium phosphate ( $NH_4H_2PO_4$ ) was used to supply the same nutrient elements in the other half. Nitrogen in the amount of 1.25 gm. was added to each pot, and phosphorus was furnished in the amounts shown in Table II. The ammonium phosphate treatments were supplemented with ammonium sulphate to maintain the uniform quantity of nitrogen per pot. Plantings were made as in the former experiment, and five plants were selected from the seedlings to remain in each pot. The spraying was conducted as in experiment I. The results are given in Table II.

TABLE II.—Chlorosis of rice on calcareous soils as influenced by fertilization with ammonium phosphate and ammonium sulphate

## SPRAYED PLANTS CUT 42 DAYS AFTER PLANTING

Pot No.	Nitrogen added as—		Phosphoric acid added as—		Yield of dry matter.	Hydrogen-ion concentration of soil water at harvest.	Number of plants.	Appearance of plants.
	$\text{NH}_4\text{H}_2\text{PO}_4$	$(\text{NH}_4)_2\text{SO}_4$	$\text{NH}_4\text{H}_2\text{PO}_4$	$\text{H}_3\text{PO}_4$				
	Gm.	Gm.	Gm.	Gm.	Gm.	PH.		
1	.....	1.25	.....	0.1	4.1	7.7	5	Green.
2	.....	1.25	.....	.5	6.0	7.7	5	Do.
3	.....	1.25	.....	1.0	9.6	7.7	5	Do.
4	.....	1.25	.....	2.5	9.7	7.7	5	Do.
5	0.02	1.23	0.1	.....	4.8	7.6	5	Yellow spotted with green.
6	.10	1.15	.5	.....	4.9	7.7	5	Do.
7	.20	1.05	1.0	.....	4.4	7.6	5	Do.
8	.50	.75	2.5	.....	2.4	7.6	5	Do.

## UNSPRAYED PLANTS CUT 42 DAYS AFTER PLANTING

9	.....	1.25	.....	0.1	2.9	7.5	5	Green.
10	.....	1.25	.....	.5	3.9	7.8	5	Do.
11	.....	1.25	.....	1.0	6.9	7.6	5	Do.
12	.....	1.25	.....	2.5	5.0	7.7	5	Do.
13	0.02	1.23	0.1	.....	1.3	7.7	5	Yellow.
14	.10	1.15	.5	.....	2.9	7.7	5	Do.
15	.20	1.05	1.0	.....	2.0	7.6	5	Do.
16	.50	.75	2.5	.....	.9	7.6	5	Do.
17	.....	1.25	.....	.....	4.0	7.7	5	Green.

## SPRAYED PLANTS CUT 77 DAYS AFTER PLANTING

18	.....	1.25	.....	0.1	60.1	7.2	5	Green.
19	.....	1.25	.....	.5	52.8	7.3	5	Do.
20	.....	1.25	.....	1.0	65.2	7.3	5	Do.
21	.....	1.25	.....	2.5	65.3	7.3	5	Do.
22	0.02	1.23	0.1	.....	51.0	7.3	5	Do.
23	.10	1.15	.5	.....	55.0	7.3	5	Striped.
24	.20	1.05	1.0	.....	60.6	7.3	5	Green.
25	.50	.75	2.5	.....	47.9	7.3	5	Do.

## UNSPRAYED PLANTS CUT 77 DAYS AFTER PLANTING

26	.....	1.25	.....	0.1	59.5	7.3	5	Green.
27	.....	1.25	.....	.5	46.0	7.4	5	Do.
28	.....	1.25	.....	1.0	53.5	7.4	5	Do.
29	.....	1.25	.....	2.5	57.5	7.5	5	Do.
30	0.02	1.23	0.1	.....	33.6	7.4	5	Yellow.
31	.10	1.15	.5	.....	27.9	7.3	5	Do.
32	.20	1.05	1.0	.....	9.5	7.7	1	Do.
33	.50	.75	2.5	.....	4.3	7.7	2	Do.
34	.....	1.25	.....	.....	54.6	7.5	5	Green.

In the original plan this test was to be conducted so that results could be obtained in duplicate, but 42 days after they were sown the plants under the influence of the different treatments showed such marked differences that it was deemed advisable to cut one-half the series in order that any tendency toward recovery might not deprive the writers of data showing the effect on plant growth of extensive chlorosis. At the time of the first cutting the plants in both pots in which the treatments had been duplicated were fairly uniform in size and appearance, so the probable error of the results obtained from the first cutting is small.

The weights of the plants that were cut when they were in bloom 72 days after planting are only approximately representative of the effect of the treatment because of the extreme variability in the size of the plants in many of the pots. In general, the variation was greatest in those pots showing the most severe cases of chlorosis, and it was apparently due to the unexplained difference in the tendency of individual plants toward recovery and normal growth. In pots No. 30 and 32 the chlorotic condition was so severe that some of the plants became diseased at the bud before they made any appreciable growth. The yields recorded for these pots represent only those plants which survived and made considerable growth after recovery.

As was true in experiment I, the effect of spraying with ferrous sulphate was only partly successful in overcoming chlorosis on account of the nature of the leaf surface. That either the iron applied was diffused throughout the leaf or that the effect of the partial development of green color was to aid the plant toward natural recovery was apparent soon after the first series of plants was cut when the spotted surface of the leaves of the remaining sprayed plants developed a striped appearance. By reference to Table II it may be seen that, regardless of the difference in fertilizer treatments, the plants that were sprayed were quite uniform in appearance, although the plants grown with ammonium phosphate were not equal in weight to those grown with ammonium sulphate.

The results obtained from experiment II show that the conditions causing the development of chlorosis were not necessarily induced by the presence of nitric nitrogen; nor were they traceable to the presence of an unassimilable basic radicle in the fertilizer salts used, in this case the indication being that chlorosis may have been caused by the precipitation of iron in the soil or in the plant by the phosphate ion liberated when the ammonium ion was assimilated at the more rapid rate.

The explanation is not definitely justified by the data at hand because it was not possible, owing to the small quantity of material available, to obtain analytical results showing the relative quantities of nitrogen and phosphorus assimilated. Moreover, it is not certain that in a calcareous soil ammonium phosphate would remain as such for any great length of time. The chlorosis which occurred in the presence of ammonium phosphate was much more severe than that which resulted from the use of sodium nitrate, but the plants grown with the former compound responded much better to spraying with ferrous sulphate and apparently tended toward a more complete recovery in the later stages of growth than did those grown with sodium nitrate.



**EXPERIMENT III.—THE COMPARATIVE EFFICIENCY OF NITRATE AND AMMONIUM NITROGEN AS FERTILIZER FOR RICE WHEN THE INTERFERENCE OF CHLOROSIS IS DIMINISHED**

In experiment I it was found (1) that the application of sodium nitrate to rice in a calcareous soil was attended by a chlorotic condition of the rice plant, and (2) that spraying with a solution of an iron salt was only partly effective in remedying the condition.

To determine, then, the comparative suitability of nitrate and ammonium nitrogen on this soil, an attempt was made to take advantage of certain individual rice plants which are less affected by the conditions causing chlorosis than are others, and also of a characteristic of the variety of rice used, the characteristic being that the rice tillers freely and produces culms and yields which are not closely dependent upon the maintenance of a definite number of plants of uniform size per unit area.

Eighteen pots were prepared and treatments were added in the same manner as they were in the previous tests, 2 gm. of nitrogen being furnished each pot in the forms of calcium nitrate, and ammonium sulphate. Calcium nitrate was chosen in preference to sodium nitrate to minimize, if possible, the intensity of the effect of the basic ion. Ammonium nitrate was used as a physiologically neutral form of nitrogen intermediate between the nitrates with basic residues and ammonium sulphate with acidic residues.

Potassium sulphate in sufficient quantities to furnish 2 gm.  $K_2O$  and phosphoric acid in quantities to supply 4 gm.  $P_2O_5$  were added to each pot. Nitrogen treatments were replicated in six pots so that cuttings might be made at different stages of plant growth. It was planned to make the first cutting of plants representing each treatment at about the time that the plants which had received calcium nitrate were showing evidence of recovery from chlorosis, selecting for samples of the rice grown with calcium nitrate the plants in those pots in which chlorosis was most severe, so that those which were least affected and which might make the quickest recovery could be allowed to mature to afford a better comparison of the yields as governed by the efficiency of the different forms of nitrogen.

The second cutting was made when the heads were beginning to emerge from the boot, at which time all the plants in the test showed a remarkable uniformity in degree of maturity.

Seeding was made as in the other experiments. Ten seedlings were allowed to remain in each pot until the pots which had received calcium nitrate could be arranged in the order of their intensity of chlorosis. Two pots representing each treatment were then selected to be held for the first cutting, 10 plants being allowed to remain in each pot, and 4 seedlings were removed from each of the other six pots. Table III gives the results of the experiment.

TABLE III.—Effect of nitrogenous fertilizers on the appearance and yield of rice plants at three stages of growth

[illegible]



In this test the rice that was fertilized with calcium nitrate was more or less chlorotic in all pots until after the first cutting was made. The plants that were furnished with ammonium nitrate were chlorotic for only the first few days of their growth. Plants that were fertilized with ammonium sulphate were not chlorotic at any stage of development.

There was a wide variation in the degree of chlorosis of the different plants which had been made chlorotic as a result of fertilizer treatment. It was therefore found advisable in the first cutting to include with the two pots treated with calcium nitrate a third pot in which the chlorosis was so severe that it was doubtful whether the plants would survive for a later cutting. At the time of the second cutting some of the plants in pots No. 4 and 5, which had been given calcium nitrate, had become green, and the others were only partly chlorotic. The plants from each of these pots were therefore divided according to their color into two samples to be weighed and analyzed.

As was found in experiment I, ammonia nitrogen was apparently almost completely absorbed by the soil very soon after the addition of the ammonium sulphate, and nitrites were not found in significant quantities at any time. This may have been due to the nonproduction of the latter form of nitrogen, to its rapid assimilation by the plants, or to conversion to other forms of such amounts as were produced.

The concentration of nitrates in the soil water had decreased considerably at the time of the first cutting, and when the second samples were taken the waters were nearly free of nitrates—a phenomenon which was coincident with the recovery of a majority of the plants from chlorosis and which may have been indirectly a contributory factor influencing recovery.

In regard to the average yield of mature heads per pot, the pots which were treated with calcium nitrate were practically equal to those which received ammonium sulphate, while the yield from the pots to which ammonium nitrate had been added was somewhat lower than that made by the others. The wide differences in yields from individual pots that were treated with ammonium nitrate may indicate, however, that the yield was influenced by some detrimental factors which did not cause other visible symptoms of injury; and it is possible that the relative inefficiency of the form of nitrogen used was not the cause of the low average yield.

The basis of selection of the pots in which the soil had been treated with calcium nitrate and from which the plants were to be harvested at maturity is open to criticism. It is thought, however, that the three pots in which the plants made rapid and complete recovery from chlorosis are comparable with the same number of pots of any other treatment, and that they are, for purposes of determining the influence of the fertilizer on the yield, more truly representative of the treatment than would have been an average which included some chlorotic plants. With this point in view it is evident that, under the conditions of this experiment, the nitrates, as represented by calcium nitrate, served for the nutrition of the rice plant as suitably as did ammonium sulphate when the factors causing chlorosis were inoperative.

#### ANALYTICAL DATA

As to the cause of chlorosis in the series of pots which received calcium nitrate, some interesting analytical data showing some of the differences between plants of the first and second cutting in experiment III and between green and chlorotic plants of the same cutting are given in Table IV.

TABLE IV.—Composition of rice plants as influenced by nitrogenous fertilizers

40 DAYS AFTER PLANTING

Pot No.	Dry matter per pot.	Increment dry matter per pot.	Nitrogen.	Nitrogen in increment.	Iron.	Iron in increment.	Ash.	Ash in increment.	Silica.	Silica in increment.	Silica-free ash.	Silica-free ash in increment.	Iron in ash.	Iron in increment of ash.	Iron in increment of silica-free ash.
	Gm.	Gm.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
1.....	7.1	.....	3.54	.....	0.023	.....	19.05	.....	11.78	.....	7.27	.....	0.1171	.....	0.3067
2.....	6.1	.....	3.59	.....	0.0175	.....	21.10	.....	13.46	.....	7.74	.....	0.0820	.....	0.2962
3.....	2.2	.....	.....	.....	0.0335	.....	26.72	.....	19.84	.....	6.88	.....	0.1246	.....	0.4840
Average..	5.1	.....	3.56	.....	0.0220	.....	20.96	.....	13.55	.....	7.40	.....	0.1049	.....	0.2973
9.....	14.3	.....	3.33	.....	0.0294	.....	17.48	.....	9.85	.....	7.63	.....	0.1680	.....	0.3853
10.....	15.5	.....	3.38	.....	0.0342	.....	17.45	.....	9.77	.....	7.68	.....	0.1960	.....	0.4453
Average..	14.9	.....	3.36	.....	0.0319	.....	17.46	.....	9.81	.....	7.66	.....	0.1827	.....	0.4162
17.....	23.0	.....	2.90	.....	0.0331	.....	17.13	.....	8.61	.....	8.52	.....	0.1932	.....	0.3885
18.....	23.2	.....	3.09	.....	0.0345	.....	17.05	.....	9.00	.....	8.05	.....	0.2023	.....	0.4286
Average..	23.1	.....	3.00	.....	0.0338	.....	17.09	.....	8.81	.....	8.28	.....	0.1978	.....	0.4082

93 DAYS AFTER PLANTING

Pot No.	Dry matter per pot.	Increment dry matter per pot.	Nitrogen.	Nitrogen in increment.	Iron.	Iron in increment.	Ash.	Ash in increment.	Silica.	Silica in increment.	Silica-free ash.	Silica-free ash in increment.	Iron in ash.	Iron in increment of ash.	Iron in increment of silica-free ash.
	Gm.	Gm.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
4.....	6 28.4	.....	0.81	.....	0.0190	.....	14.43	.....	8.74	.....	5.69	.....	0.1317	.....	0.3359
4.....	8 10.8	.....	0.81	.....	0.0184	.....	18.68	.....	13.10	.....	5.58	.....	0.0987	.....	0.3297
5.....	6 28.8	.....	0.86	.....	0.0161	.....	13.00	.....	7.67	.....	5.33	.....	0.1238	.....	0.3021
5.....	3 3.4	.....	0.95	.....	0.0208	.....	22.57	.....	15.63	.....	6.94	.....	0.0922	.....	0.2997
Average..	45.7	40.6	0.84	0.36	0.0174	0.0169	14.47	13.66	8.94	8.36	5.53	5.30	0.1202	0.1237	0.3146
11.....	62.2	.....	0.79	.....	0.0128	.....	14.93	.....	10.26	.....	4.67	.....	0.0857	.....	0.2941
12.....	48.6	.....	0.93	.....	0.0110	.....	15.14	.....	10.63	.....	4.51	.....	0.0726	.....	0.2439
Average..	55.4	40.6	0.85	(c)	0.0120	0.0047	15.02	14.09	10.42	10.62	4.60	3.47	0.0799	0.0334	0.2609
19.....	67.5	.....	0.90	.....	0.0138	.....	15.02	.....	10.17	.....	4.85	.....	0.0919	.....	0.2846
20.....	67.7	.....	0.84	.....	0.0127	.....	14.71	.....	10.03	.....	4.68	.....	0.0863	.....	0.2714
Average..	67.6	44.5	0.87	(c)	0.0132	0.0025	14.86	13.70	10.10	10.77	4.76	2.93	0.0888	0.0188	0.2773

a Green plants.

b Chlorotic plants.

c Loss.

The samples analyzed represent the entire plant above ground. The dry matter produced under the influence of each treatment was found to vary considerably, especially in those pots which received calcium nitrate, and the results of the analyses are therefore weighted in proportion to the amount of dry matter produced in the respective pot for the calculation of the average.

#### ANALYTICAL METHODS

The analytical methods used were essentially those prescribed by the Association of Official Agricultural Chemists.<sup>3</sup>

Nitrogen was determined by the Gunning method, sodium sulphate being used in place of potassium sulphate. In order to determine the ash and its constituents, the material was charred over a low flame, the sample was weighed, leached with dilute hydrochloric acid, and then filtered and washed on a tarred Gooch crucible having a pad of ash-free filter paper. The insoluble residue consisting of silica and carbon was dried and weighed and the carbon was burned off. The ash percentages were calculated from the weight of the original incinerated material by subtracting therefrom the loss of weight due to the ignition of the carbon.

Carbon dioxid in the ash was not determined on account of the small quantity of material available. The quantity of silica reported is the sum of the weights of insoluble silica and of that in the hydrochloric acid extract which was obtained by evaporation and dehydration. Iron was determined by permanganate titration after it was reduced with zinc and by the Reinhardt method. The results obtained by each method were in agreement.

#### DISCUSSION OF RESULTS OF ANALYSIS

Table IV discloses some results which may have a positive significance in showing the influences causing the chlorosis in experiment III. Table III shows that the nitrates had practically disappeared from the soil water at the time of the second cutting, and Table IV shows that a marked decrease in the percentage of nitrogen in the plant occurred during the period between the first and second cutting, those plants which had received nitrogen as calcium nitrate having gained 0.36 per cent of nitrogen in the increment of dry matter, while the plants in the other two series showed a loss of total nitrogen.

If, therefore, the production of basic residues by the assimilation of the nitric fertilizer salts produced in the soil a condition that prevented the absorption of iron, or in the plant a condition which prevented the utilization of iron for the development of chlorophyll, the influence must have been removed before the plants in experiment III were ready for the second cutting, and at a time that was approximately coincident with the recovery of the plant from chlorosis.

A comparison of the average results of the iron determinations indicates that, in those plants which received calcium nitrate, the first cutting of the plants had less iron in the dry matter, in the ash, and in the silica-free ash than was found in the same components of the green plants grown with the other treatments. In the second cutting the chlorotic plants had the higher content of iron in the dry matter; and while the

<sup>3</sup> WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig., 1908. Reprinted 1912.

percentage of iron in the ash was higher in the green plants, this was largely due to a high proportion of silica in the ash of the chlorotic plants. The percentage of iron in the silica-free ash was about the same in the green and in the chlorotic plants.

Such observations would indicate that the chlorosis in these pots was not due to a deficiency of iron, but Gile and Ageton (7) explained a similar result in their work as possibly being due to the fact that the tissues of chlorotic plants represent an earlier period of growth than do the tissues of green plants of the same age and that they therefore require a greater quantity of iron to prevent chlorosis. It would not be impossible, however, for iron in a chlorotic plant, though present in sufficient quantity, to be rendered inactive for the development of chlorophyll by abnormal conditions in the plant.

It is shown that the green plants had an ash content which was less than that of the chlorotic plants, but the high ash content of the latter may have been due to the depression of carbon assimilation and to a consequent small quantity of carbonaceous matter in the plant. The high silica content of the chlorotic plants can best be explained in the same way, and variations in the amount of this constituent in the plant ash are apparently of no importance.

In regard to the silica-free ash, the plants of all treatments had a general uniformity of compositions at the same stage of growth but gave some evidence that the use of calcium nitrate as a fertilizer depressed the absorption of this constituent up to the time of the second cutting. It is hardly possible, however, that the silica-free ash content of the plant can be considered as indicative of the way in which nitrate fertilizers cause the development of chlorosis, because the analysis of plants in the second cutting from the nitrate pots showed that there was no consistent difference between the chlorotic and the green plants in this regard.

As was shown of the nitrogen content, the rate of absorption of the ash constituents, with the exception of silica, tended toward diminution as the plant passed through the stage in which it began to recover from the condition causing chlorosis. It does not appear, however, that the analytical results presented offer an evidence that variation in the ash content of the plant at different periods of growth has any bearing on chlorosis.

#### SUMMARY

The development of chlorosis of rice on calcareous soils may be governed by the nature of the nutrients supplied, those compounds represented by sodium nitrate, calcium nitrate, and ammonium phosphate, which, in themselves or by virtue of an unassimilable ion, are the cause of the precipitation of iron, being associated with chlorosis, while the plants which have been supplied with ammonium sulphate in which the unassimilable radicle may serve as a solvent for iron may be thrifty and of normal color.

It has not been shown by the experimental methods used whether the basic material in the soil or the basic residues of the nitrates were primarily the cause of the conditions determining the appearance of chlorosis, nor whether the acidic residue of ammonium sulphate served to prevent chlorosis in the calcareous soil. Inasmuch, however, as it was shown in the first experiment that the degree of chlorosis attending the use of sodium nitrate as a nutrient was in proportion to the quantity

of that salt furnished, it seems possible that the basic residues of the nitrates were primarily the cause of the chlorosis and that the alkalinity of the soil was a secondary factor.

The reaction of the soil in which the plant roots are in contact with the insoluble supply of iron is shown to be of less significance in determining the availability of iron for the plant than are the effects of the products rejected by the plant roots.

Chlorosis of rice grown on the soil used was especially severe in young plants, and plants which were not too seriously affected showed a tendency to recover as they approached maturity. This phenomenon of recovery was coincident with a decrease in the rate of absorption of nitrogenous compounds, a fact which substantiates the evidence that the nitrate fertilizers were causative of the chlorotic condition, although it is possible that an increase in the elimination of carbon dioxide from the roots had some influence in aiding the plant to recover its normal color by making more iron available in the soil.

The observation that nitrates are less suitable than ammonium salts for the fertilization of young rice plants may be based on the influence on the plants at the period of greatest absorption of nitrogen of the unassimilated residues of the fertilizer used rather than on the inferiority of nitric nitrogen as a nutrient. It seems probable also that the injury which has heretofore been ascribed to the toxic effect of the nitrites, derived from nitrates by reduction, was but a manifestation of chlorosis which was caused by the action of the basic residues of the nitrate salts used as nutrients.

The nitrogen of calcium nitrate may be suitable equally with that of ammonium sulphate in the physiological processes of the rice plant when it is used under cultural conditions in which the reaction of the unassimilated residue of the nitrate does not interfere with the absorption and utilization of iron.

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## SOME GRAMINICOLOUS SPECIES OF HELMINTHOSPORIUM: I<sup>1</sup>

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### INTRODUCTION

Although the genus *Helminthosporium* includes a large number of forms thriving saprophytically on the bark, leaves, and stems of both woody and herbaceous plants, it has become familiar to plant pathologists and perhaps, in a large measure, to students of fungi generally, through a relatively moderate number of parasitic species. Undoubtedly the most widely known of these parasitic forms are those affecting graminaceous hosts, as considerable losses to important cereal crops, including especially barley, corn, rice, oats, wheat, and sorghum, in various parts of the world, have continued for several decades to encourage a desire for knowledge leading to some sort of effective control. Besides these economically important forms, many parasitic species of *Helminthosporium* have been recorded as thriving on various members of the grass family, but have remained more or less obscure because either the hosts affected were of little economic value, or, being important, the parasitism occasioned little or no observable damage.

However, as is not uncommon in the case of large genera, publication of an increasing number of descriptions of presumably new species, thriving on related or even identical hosts, has injected a large degree of uncertainty into the specific taxonomy. In many instances, writers have failed to compare their organisms with congeneric forms, or have used for such comparison herbarium material which had already undergone the degenerative changes in structure incident to the death of the spores. It was in an effort to define the more distinctive differences between the forms parasitic on barley and oats, and those found on a few of the more common uncultivated or wild grasses that the present study was undertaken. This paper, which it is hoped may be followed by others dealing with the very considerable variety of species of *Helminthosporium* growing on grasses in the United States, is offered as a comparative mycological account of some of the more readily available species. No attempt is made here to deal with the intimate pathological aspects, as these have been for some years the subject of intensive study by other workers, both in this country and abroad.

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## APPEARANCE OF AFFECTED PLANTS

Although the symptoms produced by a species of *Helminthosporium* on some particular host are generally quite well defined, the different members of the genus considered collectively bring about a considerable variety of changes. These may be briefly considered under a number of categories.

## SPOTBLOTCH, FOOTROT, EYESPOT

Perhaps the most easily recognized type of lesion is represented by the discoloration resulting from the attack, for example, of *Helminthosporium sativum* P. K. & B. on barley (*Hordeum* spp.), wheat (*Triticum* spp.), and quack grass (*Agropyron repens* [L.] Beauv.). Here each foliar infection produces a fairly well-defined, more or less longitudinal spot ranging in color from light brown to nearly black. An entirely similar type of leaf injury is characteristic of the diseases of Kentucky bluegrass (*Poa pratensis* L.) and of barnyard grass (*Echinochloa crus-galli* [L.] Beauv.), attributable to two congeneric parasites that are newly described in this paper as *H. vagans* and *H. monoceras*, respectively. When the leaf sheaths also are affected, the discoloration often becomes increasingly diffuse downward, so that the base of the stem may be quite uniformly discolored—a condition that in the case of wheat, where it is usually complicated with more or less injury to the roots, has become widely known as footrot. Similar dark foliar spots characterize the incipient attack of *H. leersii* Atk. on white grass (*Leersia virginica* Willd.), of *H. giganteum* H. & W. on goose grass (*Eleusine indica* [L.] Gaertn.), and Bermuda grass (*Cynodon dactylon* L.), and of *H. oryzae* B. de H. on rice (*Oryza sativa* L.). Later, however, the central areas lose their dark color, the older and larger spots being finally represented by a dark brown ring surrounding a central straw-colored area. The term "eyespot," which has been applied (73)<sup>8</sup> to a disease of sugar cane (*Saccharum officinarum* L.) caused by *H. sacchari* Butl., is perhaps most accurately descriptive of the latter type of foliar lesions.

## NETBLOTCH

A second category of discoloration very characteristic, though less common, is caused by *Helminthosporium teres* Sacc. on barley, as well as by a fungus on *Festuca elatior* L. described in this paper as *H. dictyoides*. The affected leaves, while still green and living, show abundant brownish discoloration in irregular pattern, within which may be recognized a network of darker longitudinal and transverse linear streaks. With the withering of the leaf these reticulate markings become less pronounced, and are finally more or less completely obliterated.

## STRIPE

*Helminthosporium gramineum* Rabh. causes an unusual type of injury to barley, the leaves previous to heading time becoming variegated with yellow bands extending frequently the whole length of the leaf. On the premature death of the plants the foliar organs split along longitudinal markings, giving them a ragged or shredded appearance. This type of injury has been shown to be contingent on continued development of the parasite in the growing tissues in a manner unlike the development of certain smut fungi.

<sup>8</sup> Reference is made by number (italic) to "Literature cited" pp. 737-739.

## WHITE BLAST

*Helminthosporium turcicum* Pass. produces on maize (*Zea mays* L.) a type of injury that is probably more common than might be supposed, as it is likely to escape detection. The green color of the affected tissue disappears completely, leaving a chlorotic area that increases in size until it may be several inches long and perhaps one inch in width. Owing to the large size of the corn leaf blade, the desiccated areas, which may be surrounded, moreover, by a narrow, brownish, marginal zone, contrast sharply with the surrounding green tissue and are quite readily recognizable as due to the agency of a parasite. However, with the larger number of graminaceous hosts, having much smaller leaves, proportionately large segments of the blade are involved at once; withering usually starts at the tip and proceeds downward, thus simulating the appearance of withering due to drought. It may be mentioned that the parasitic nature of species of *Helminthosporium* not associated with dark discoloration or conspicuous pathological changes in the mechanical properties of the plant tissues involved manifestly can not be ascertained definitely by observation alone. *Helminthosporium dematioidicum* Bub. and Wrob. on the leaves of sweet vernal grass (*Anthoxanthum odoratum* L.) may be cited as an example of a considerable number of fungi, the relation of which to their host or substratum is certainly not as obvious as might be desired.

## OTHER TYPES OF INJURY

Differing quite markedly from all of these forms of injury is that caused by a species of *Helminthosporium* attacking young plants of a species of *Paspalum*, provisionally identified as *Paspalum boscianum* Fluegge, which will be further discussed as *H. micropus*. The first evidence of infection appears as a water-soaked area, the tissue of which has lost all its rigidity. The condition suggests an injury such as might have been brought about by the application of a few drops of boiling water. The affected area frequently dries and shrivels, often leaving the surrounding tissue quite normal; or if the affected spot is large enough to interrupt the vascular communications the more distal portion of the leaf blade may gradually wilt without any further advance of the fungus.

In the attack of *Helminthosporium ravenelii* Curtis on *Sporobolus indicus* (L.) R. Br. it is quite impossible to notice either wilting or discoloration. The fructifications of the fungus grow directly out of the inflorescence in a dense, dark, brownish green, velvety layer, the latter being so conspicuous and abundant as to have suggested the term "smut grass" as common name for the host. The infection undoubtedly is altogether local, for even when the larger portion of the panicle has been overgrown with the fungus, the exposed parts present an appearance not greatly different from that of an entirely healthy inflorescence. The fungus discussed in this paper as *H. oryzae*, when developing on the inflorescence of rice, shows an approach to *H. ravenelii* in the crowded mass of its sporophores.

## TAXONOMIC CHARACTERISTICS

The definition of the genus *Helminthosporium* Link (85) as recognized in the large works of Saccardo (128, v. 4, p. 402) and Lindau (83) has been very generally adopted by mycologists. (The genera *Brachysporium*

Sacc. (128, v. 4, p. 403) and *Napicladium* Thüm. (150) are distinguished from *Helminthosporium*, the former in possessing relatively short spores, the latter in having spores tapering toward the apex.) Naturally, these distinctions are based on no fundamental differences, and may readily be supposed to have been advanced primarily to serve in dividing in some way a large group of organisms into a number of smaller groups. As it is not always possible to determine definitely whether the spores of a certain species are in the main to be regarded as longish, or short, or tapering, the advantage accruing from erection of these genera on artificial distinction has been at least partly counteracted by the confusion occasioned by different writers assigning the same form to different genera. Indeed, certain of the forms included in the present paper have been assigned by some writers to one or the other of the genera allied to *Helminthosporium*, and a few other forms might perhaps be thus assigned with equal cogency.

The imperfect fructifications characteristic of the genus *Helminthosporium* consist of sporophores emerging from the substratum singly or in clusters, or more rarely arising as a dense, velvety layer. After attaining a certain length and becoming usually one-to-several-septate, depending somewhat on the species, a spore is developed at the tip. After the latter has reached a certain degree of maturity the sporophore continues to grow near the point of attachment of the first, pushing aside the first spore, and producing another spore a little farther on. A number of spores are thus produced, which, with the exception of the last one, usually fall off or are blown off in nature, their places of attachment being marked by a series of geniculations bearing a dark scar at the apices. In most foliicolous species, the sporophores as collected in the field show branching in only relatively infrequent instances, although a few forms exhibit a more readily demonstrable tendency toward ramification. In *H. ravenelii* branching of the sporophore is, however, very abundant and accounts in a measure for the dense, velvety texture of the crust. Length, width, color, frequency of septation, and general habit of the sporophore, while of considerable value in the diagnosis of some species and consequently always to be given consideration in specific descriptions, in general leave much to be desired in distinctiveness.)

The mode of emergence from the epidermis of the host is usually not especially characteristic. In the case of those grasses having small leaves provided with a relatively delicate epidermis emergence usually takes place in an altogether miscellaneous manner, either from the stomata or between epidermal cells. In the case of those grasses of which the epidermis is mechanically somewhat stronger, as in barley or barnyard grass, the sporophores, especially in the beginning before the leaf tissue becomes distorted by shriveling, show a tendency to emerge from the stomata, although emergence between epidermal cells is never uncommon. The only instance observed by the writer where the sporophores appear to be entirely confined to the stomata is that of *Helminthosporium turcicum* on corn leaves, the localization here being readily attributable to the character of the epidermis, which in this host is of unusual mechanical strength, the stomata on the other hand being large and uniformly distributed. Sporophores may appear singly, or in clusters ranging in numbers from 2 to 5, 6, or even 7. Small clusters usually are proliferated nearly simultaneously. However, where the number runs up to half a dozen, some of the sporophores are usually younger,

having been developed after the first two or three had perhaps commenced to proliferate spores.

By far the most distinctive characteristics for taxonomic purposes are to be found in the spore. Within the genus these structures show an unusual range of difference with regard to size. Those of *Helminthosporium giganteum*, for example, which are among the largest reproductive bodies produced by any group of fungi, exceed those of the smaller species like *H. dematioideum*, about twice in width and ten times in length, although the latter can not be regarded as especially small. The length of the spore, particularly in the case of those species in which this body is nearly colorless and straight cylindrical like *H. tritici-repentis* Died. *H. bromi* Died., *H. teres*, and *H. giganteum*, is subject to great variation, the length of the smaller ones often scarcely exceeding their width. For this reason (the magnitudes approaching the maximum are to be regarded as the more characteristic of the species) (The width of the spores of any particular species on the other hand is much more apt to be nearly uniform, regardless of considerable differences in length.)

With respect to coloration, the spores represented in the genus exhibit all gradations between the subhyaline condition present, for example, in *Helminthosporium giganteum* and *H. tritici-repentis*, to the dark olivaceous hue characteristic of *H. sativum* and a considerable number of closely related congeneric forms. Most of the species are intermediate between these extremes—yellowish, yellowish brown, and brown. A few species have spores with subhyaline end cells, all the other cells being uniformly dark; or the middle cells may be dark, with the color becoming perceptibly fainter toward the ends.

The spores of most forms of *Helminthosporium* are characterized by some peculiarity in shape by which they can be often recognized with a high degree of certainty. The general contour may be straight cylindrical as in *H. avenae* Eidam, curved ellipsoidal as in *H. sativum*, or tapering toward the tip as in *H. dictyoides*. The basal end is usually most characteristic. In *H. teres*, the proximal cell is hemispherical; in *H. bromi*, hemi-ellipsoidal; in *H. tritici-repentis*, elongate conical with rounded apex, thus crudely suggesting in outline the top or bottom aspect of a snake's head. In *H. monoceras*, the basal half tapers gradually toward the basal end, which is abruptly rounded off; in *H. turcicum* the tapering is more abrupt and continues to the insertion of the hilum. The same is true in *H. micropus* except that here the extreme basal end is drawn out somewhat, the contour being slightly concave near the hilum. In this connection, it may be stated that the hilum—that is, the more or less calloused region at the base of the spore, marking the place of attachment to the sporophore—may be represented by a conspicuously protruding modification as in the two species last mentioned; or, as is much more frequently true, it is visible as a dark spot situated altogether within the contour of the spore wall. In a few species the hilum is not at all conspicuous, as in *H. giganteum*, where it appears as a small wedge-shaped basal prolongation, with a delicate single-contoured confining membrane.

As a good deal of the taxonomic literature based on dead herbarium material gives a wrong impression of the structure of the spore, particularly with reference to its walls and septa, a few remarks may be justified here. The developing spore remains nonseptate during the earlier stages of growth, usually until its definitive size has been largely

attained. Cross walls then appear, the first formed segments being further reduced in size by later successive divisions. Although at the time a segment has just undergone division, the appearance under the microscope is that of a thin line extending across the spore, the protoplasts thus delimited soon round up along the zones where cross walls are in contact with the external wall. The latter in the hyaline-spored forms seems to undergo no readily perceptible increase in thickness; in the dark-spored forms the subsequent apparent increase in thickness is quite considerable. In the process of maturation the individual segments appear to deposit a membrane of their own, the matured spore thus consisting of the original outer spore wall, inclosing a row of more or less independent segments, like peas in a pod. In some species, as for example, *Helminthosporium leersii*, where the outer wall is relatively delicate, these segments may be removed readily from their enveloping membrane by tapping or gently pressing on the cover glass. The process often results in no injury at all, each segment being capable of germinating independently and promptly.

— The mode of germination characteristic of a species usually bears a more or less apparent relation, both to the shape of the spore and the structure of its wall. In most of the forms with cylindrical hyaline spores, where the peripheral wall is everywhere uniformly thin, germ tubes are produced more or less indiscriminately from all segments, basal, apical, and intermediate. In the species with tapering spores, as *H. dictyoides*, for example, one germ tube usually arises from the distal cell, and one or two from the larger basal cell, the intermediate segments rarely participating directly in the process.<sup>4</sup> The germ tubes are not polar in position, but arise from undifferentiated regions usually laterally or obliquely at some distance from the hilum or the extreme apex. The fully matured spores of *H. sativum* and closely related species have two perceptibly thin places in the peripheral wall, these being located at the tip and at the base of the spore, in the latter case occupying a narrow zone adjacent to and surrounding the hilum. These areas may be clearly seen, for example, in *H. monoceras*. Here normal germination takes place by the production of one germ tube from the thin-walled region at the tip, or from that near the hilum, or more often from both; never from the intermediate segments, unless, as has been mentioned, the latter have been partly exposed or completely liberated from the enveloping wall by manipulation or mechanical injury. In some other forms like those later discussed under the names *H. halodes*, *H. rostratum*, and *H. oryzae*, germination of entirely mature spores takes place as in *H. sativum* and *H. monoceras*; but not infrequently newly proliferated, subhyaline spores, the walls of which have not become thickened, can be seen to produce, in addition to the two polar germ tubes, one or more tubes from the middle cells as well.

A modification of the septa, visible in the spores of some species, and undoubtedly present in others, may be not without significance in germination. If the cross walls of large, subhyaline-spored forms, like *Helminthosporium teres* or *H. giganteum*, are closely examined a small circular spot having perhaps one-fifth or one-fourth the diameter of the spore may usually be distinguished. It is difficult to determine whether this represents an open communication between the segments or merely two opposed pits in the adjacent segment walls. The appearance of these walls in plasmolyzed dead spores would suggest that the latter alternative is the more probable one, a suggestion that is supported by the fact that

each segment suffers death independently of the others. There can be little doubt, however, that this modification provides means of communication between adjacent living segments. Such a communication would manifestly be of importance especially in those species in which germ tubes are produced only from the two end cells. There are, it may be mentioned, peculiarities in the germination of some species that can not be definitely ascribed to the structure of the septa or of the peripheral wall, or indeed to any demonstrable structural feature; as, for example, the production of three germ tubes from the basal cell of *H. dematioideum*, or the proliferation of clusters of tubes from the end cells or middle cells in *H. giganteum*.

It is interesting to note that if leaves bearing actively sporulating fructifications of forms possessing thin-walled, hyaline spores, like *Helminthosporium teres* or *H. bromi*, are examined under the microscope by reflected light, it will be seen that the mature spores lying about in some abundance are badly distorted and their walls utterly collapsed. When these spores are mounted in water, most of them instantly become turgid, and at a suitable temperature begin to germinate within 30 minutes, showing that the collapsed condition is by no means an indication of death. Dead spores or dead segments of partly living spores, also present in a preparation, recover their former size and shape very largely, although not wholly, but are readily recognized by their abnormally swollen membranes and coarsely granular contents made familiar by the drawings and descriptions of many authors, who unfortunately regarded them as normal. Although some authors report germination of spores exhibiting such swollen walls presumably in all segments, either as a result of age or the application of reagents, the writer has never observed an instance of viability in such material.

Germination is usually accompanied by conspicuous protoplasmic changes beginning in the segments concerned, and finally involving all the segments of one spore. The contents lose their uniform hyaline structure, becoming minutely granular or uniformly vacuolate, or more usually both granular and vacuolate. The germ tubes and mycelium in general vary somewhat with the different species. All the species seem to thrive on the culture media commonly employed in laboratories, which fact, together with the large size of the spores, makes the members of the genus among the fungi most easily isolated and cultivated. Not all of the species, however, can be made to sporulate in pure culture, those possessing hyaline spores—*Helminthosporium bromi*, *H. tritici-repentis*, etc.—being especially refractory in this respect. The majority of forms with dark spores, on the other hand, sporulate quite readily and even abundantly, although the spores thus produced may depart somewhat in shape and size from those produced under ordinary field conditions. This is particularly true of *H. sativum*, where the spores, instead of being long and slender-allantoid, become shorter, thicker, and nearly straight. When media containing a large amount of nutrient is employed, an abundant development of mycelium usually takes place and sporophores are produced in great numbers. (Growth, however, soon comes to a standstill, leaving the sporophores short, and bearing only a few spores, the latter frequently being abnormally short. In such cases more satisfactory results can be obtained by the use of media containing little nutrient, as corn-meal-decoction agar or tap-water agar. If such cultures are protected from evaporation, the relatively small number of sporophores will continue to grow for several months, producing scores of



fairly characteristic spores in a dense racemose cluster. Sporophores thus produced may show a tendency to branch, not usually observable in material collected in the field.

A process quite analogous to germination may be observed in the proliferation from the spores of *Helminthosporium gramineum*, of short sporophores in the regions where ordinarily germ tubes appear. These bear a variable number of secondary spores that may usually be distinguished from the primary spores by their smaller dimensions. When this takes place while the latter are still attached to the sporophore, the whole apparatus may bear a partial resemblance to an *Alternaria* fructification. A somewhat similar condition appears to obtain quite normally also in *H. catenarium*, the fungus parasitic on wood reedgrass (*Cinna arundinacea* L.), where, however, the secondary spores more often are borne directly at the tip of the primary one, which thus comes to bear two hila. When grown in culture, this species develops ramifying fructifications in which a series of spores alternating with short sporophoric hyphae bear short sporophoric processes from the end cells. In fructifications of this type, the distinction between sporophore and spore is at least partly obliterated, the two types of structures merging into one another. This miscellaneous type of growth may be observed when *H. teres*, *H. cyclops*, and even *H. sativum* are grown on artificial media. This growth is apparently encouraged by moist conditions, although, as has been indicated, it appears to occur in nature in the case of *H. catenarium* and *H. gramineum*.

It may not be out of place to mention a peculiarity in the growth of the mycelium of most species of *Helminthosporium* which, although perhaps not confined to this genus, may not be without significance. If, for example, a number of spores of *H. bromi* are mounted in water and two come to lie in contact with each other, it will be seen that usually one or more pairs of germ tubes are proliferated from approximately opposite positions and immediately anastomose, thus uniting the two spores by several short hyphal connections. (Pl. 8, Dc.) This tendency toward anastomosis is expressed even more strongly in the mycelium that develops somewhat below the surface of agar cultures, and may be studied conveniently by cultivating the fungus on poured plates, the anastomosis occurring abundantly near the lower surface of the agar. Some of the cells involved in these hyphal fusions swell into subglobose bodies and proliferate short irregular processes of inflated segments, the whole resulting in small, dark brown, knotty masses of mycelium. Some of these continue to increase in size, developing into the subspherical sclerotia, readily visible to the naked eye, the profuse occurrence of which in culture is a distinguishing mark of this species. Although the writer has not succeeded in cultivating these sclerotia further, there can be little doubt that they represent immature perithecia, as they are entirely similar to the immature perithecia found on leaves of the natural host in fall. The inference is further strengthened by the fact that other species of *Helminthosporium*, *H. teres* and *H. tritici-repentis*, of which ascigerous conditions are known, show these structures in abundance. The writer is inclined to believe that in whatever species such sclerotia or abundant anastomoses (resulting in the production of complexes of lobulate segments) are found to occur, perithecia may be sought with considerable prospect of success.

Ascigerous forms have been reported for a relatively small number of species of *Helminthosporium* parasitic on grasses. These are all readily referable to the genus *Pyrenophora* Rab. or to *Pleospora* Fries, depending on the presence or absence of bristles on the perithecium—a basis for generic distinction, which, while regarded by Winter (159, p. 493) with considerable justification as inadequate, has been recognized as valid by Saccardo (128, v. 2, p. 238) and Lindau (82, p. 429). *Helminthosporium bromi*, *H. tritici-repentis*, and *H. teres* have associated with them in this country ascigerous forms belonging to the genus *Pyrenophora* that occur in great abundance on dead host material in spring, *Pyrenophora teres* (Died.). (= *Pleospora teres* Died.) being found on the spike and culm of barley straw, *Pyrenophora tritici-repentis* (Died.) (= *Pleospora tritici-repentis* Died.) on the culm, and in slight measure on the decaying leaves, of quack grass, while *Pyrenophora bromi* (Died.) (= *Pleospora bromi* Died.) is very abundant on the leaves of awnless brome grass, *Bromus inermis* L. In the latter two species the perithecia usually reach perfect development, asci and ascospores reaching maturity early in May near Madison, Wis. Material collected at that period exhibited abundantly the preliminary swelling and circumscissile rupture of the ascus preceding the simultaneous expulsion of the spores from near the base of the dehiscing structure—a mode of discharge observed by Porter (110) in species of *Pleospora*, and in the species under consideration by Diedicke (28) as well as by Atanasoff (2).

*Pyrenophora teres*, on the other hand, probably does not form altogether normal ascospores, if the observations made in the spring of 1919 and 1920 may be taken as typical. The ascus may remain undeveloped, showing no trace of young ascospores; or ascospores may be delimited, but become arrested in their development before or after septation has occurred; or certain segments of the spore may develop normally, the others eventually collapsing, giving rise to the unsymmetrical spores shown in Plate 3, D. As Diedicke (29) and Noack (95) have shown, the developing perithecia or sclerotia begin to proliferate conidiophores with the advent of suitable conditions; and a perithecium involved in the production of conidiophores is not likely to show any further development of its ascospores. The production of conidia usually is large in the case of the sclerotial form on barley; moderate in the case of *Pyrenophora tritici-repentis*; and small in the case of *Pyrenophora bromi*. The latter species is probably the only one in which the production of ascospores plays an essential part in the resumption of growth in spring; for, as a rule, in the forms on quack grass and barley, the conidia produced on the sclerotium or immature perithecium would appear to play a relatively larger part in effecting early dissemination on the young host plants. In the case of the forms with dark, thick-walled spores, as for example, *H. sativum* and *H. vagans*, with which neither sclerotia nor perithecia are known to be associated, the conidia are found to germinate readily in spring, since exposure in winter does not bring about any very decided diminution in viability. Conidia of *H. sativum*, *H. oryzae*, and *H. ravenelii* will germinate well one year after their formation, whereas those of *H. bromi*, *H. tritici-repentis*, and *H. teres* are mostly dead within one or two months. The absence of an ascigerous stage from the life history of the former species, and its presence in the latter, are probably not without significance in relation to the longevity of the conidia.)

## HELMINTHOSPORIUM GRAMINEUM RAB.

*Brachysporium gracile* (Wallr.) Sacc. var. *gramineum* (Rab.) Sacc. 1886, in *Sylloge fungorum*, v. 4, p. 430.

*Napicladium hordei* Rostrup 1893, in *Sygdomme hos landbrugsplanter foraarsagede af Snyltesvampe*, p. 130-132.

*Helminthosporium gramineum* (Rab.) Erikss. 1885, in *Fungi par. scand. exs.*, no. 187.

*Helerosporium gramineum* of Oudemans, not Rabenhorst.

The binomial *Helminthosporium gramineum* was applied by Rabenhorst<sup>4</sup> to a fungus occurring on leaves of barley (*Hordeum vulgare* L.) collected at Poppelsdorf, Germany, June, 1856, and distributed as No. 332 of the Herbarium Mycologicum. The pieces of leaves and stem that constitute the specimen deposited in the Herbarium of the Office of Pathological Collections are too small to show any possible characteristic pathological effect; and the fructifications of the fungus are in a condition that would appear to make its identification with any particular one of the three congeneric species now known to occur on barley a matter of great uncertainty. Rabenhorst regarded the fungus as related to *Helminthosporium gracile* Wallr. but differing from the latter in that its spores were solitary, elongated-cylindrical, and 3 to 6 septate. Apparently, in accordance with this view, Saccardo (128, v. 4, p. 430) at first reduced the form to a variety of Wallroth's species, which, moreover, he transferred to the genus *Brachysporium*. Later, however, he listed it as an independent species (128, v. 10, p. 615).

In 1886, Eriksson<sup>5</sup> distributed as *Helminthosporium gramineum* (Rab.) Erikss. specimens of diseased barley collected near Stockholm, Sweden, during the preceding season. The label includes a short revision of the specific diagnosis:

Hyphi conidiophori solitarii vel 2-4 aggregati, subflavi 1-5 septati, denique saepe angulato anfracti. Conidia subflava, recta, elongato cylindracea, 1-5 septata, 50-100  $\mu$  longa, 14-20  $\mu$  lata.

Although this characterization applies better to the parasite causing the stripe disease than to that responsible for net-blotch, Eriksson apparently did not distinguish between the two. At any rate, the specimen in the herbarium of the Office of Pathological Collections shows lesions of both diseases; and in an account (37) of the "leaf spot disease" (*blad fläckensjukdom*) he reported most of the plants to have been affected more or less, while a relatively small proportion (1 to 5 per cent) were affected so badly that no heads were developed. It may readily be supposed that the less severely diseased plants were mainly affected with net-blotch instead of with stripe, a supposition supported by the description of the foliar lesions as elongated dark brown spots with light margins.

Von Post (111), working independently of Eriksson, in 1886 published an account of the "brown stripe disease" (*brunrandsjukdom*) of barley, that had been very destructive at Ultuna, Sweden. The longitudinal yellowish streaks, characteristic of all the leaves of attacked individual plants, suggestive of the variegation of ribbon grass, and later changing to brown or yellowish brown; the dying of the plants before the development of spikes, the systemic distribution of the fungus (indicative of seed transmissal and seedling infection) provide conclusive evidence that this investigator was dealing exclusively with the stripe disease.

<sup>4</sup> RABENHORST, G. L. KLOTZSCH HERBARIUM VIVUM MYCOLOGICUM SISTENS FUNGORUM PER TOTAM GERMANIAM CRESCENTUM COLLECTIONEM PERFECTAM. . . Ed. II, Century III, no. 332. 1856.

<sup>5</sup> ERIKSSON, J. FUNGI PARASITICI SCANDINAVICI ENUMERATI NO. 187. *Helminthosporium gran* (Rab.) Erikss. Stockholm, Sweden. July, 1886.

The conidial form that von Post observed developing on the brown areas of affected leaves he identified with *Helminthosporium gramineum* Rab. as defined and distributed by Eriksson.

Rostrup (123), in 1888, described a disease characterized by symptoms and development similar to those described by von Post, which had been doing considerable damage in certain sections of Denmark, and which he attributed to a new species of *Napicladium*, *N. hordei*.

In 1892, Pammel (102) reported a destructive disease of barley from Iowa, which obviously was identical with that described by von Post, being evidenced long before heading time by the presence on all the leaves of affected individual stools, of pale yellow streaks extending from base to tip, premature death, and subsequent tearing of the foliage into shreds. Pammel identified his fungus with the one distributed by Eriksson as *Helminthosporium gramineum* Rab.

Publications by Ritzema Bos (120) and Frank (43) are evidence that the same type of malady appeared and caused losses during the next few years in Holland and in Germany. However, Ritzema Bos' figures of the foliar lesions as well as of the 8-septate spore, and the description of the symptoms in Frank's earlier account (42) leave a suspicion that, like Eriksson, these authors were dealing not with stripe alone, but also with net blotch, and failed to distinguish between the two diseases and causal organisms.

Rostrup (125) appears to have been the first investigator to recognize that barley was affected by two different diseases caused by two distinct related fungi. However, as he associated Rabenhorst's binomial with the cause of the less destructive "leaf spot disease" (*bladpletsyge*) instead of with the "stripe disease" (*stribesyge*), and attributed the latter to a different although related genus of fungi, the prevailing taxonomic confusion was not immediately settled. Indeed, it was not until the appearance of Ravn's detailed papers (115, 116) that the ambiguity, which Eriksson's publications had originally introduced, was disposed of effectively. Ravn assigned the parasite causing stripe to *Helminthosporium gramineum* Rab., reduced *Napicladium hordei* Rostrup to a synonym, and distinguished the fungus very clearly, with regard to morphology and pathogenicity, from the congeneric forms causing net blotch of barley and leaf spot of oats.

✓ While Ravn's papers (115, 116) thus left no further occasion for confusing *Helminthosporium gramineum* with *H. teres*, it did nothing to distinguish it from *H. sativum*, a species later described from the United States, but the occurrence of which as a third congeneric form parasitic on barley has not been recognized in Europe. As will be pointed out in another connection, the European literature is not devoid of ambiguous accounts, of which Massee's treatment (90) of barley leaf blight may be taken as an example, indicating that *H. sativum* is certainly not altogether absent, but usually is mistakenly recognized as *H. gramineum*. And a similar condition obtains in the writings of investigators in America and other countries.

Yet after allowances for erroneous diagnoses are made, stripe remains one of the most important and widespread diseases of barley. In Europe it has been reported not only from Sweden (111), Denmark (115), Germany (42), and Holland (120), but also from England by Prain and Percival (112) and by Biffen (13), from Ireland by Johnson (72), and from Russia by Jachewski (66). According to the records of the Plant Disease Survey, it is found in most of the States of the Union, apparently wherever

barley is grown to any extent, the damage varying from a relatively small amount to approximately one-fifth of the crop. From Canada the disease has been reported by Güssow (48) and by Fraser (45). Hauman-Merck (52) noted the prevalence of *Helminthosporium gramineum* on barley in Argentina to an extent unknown in Central Europe. Yoshino (161) and Ideta (65) have recorded the disease in Japan, where Hori (63), in 1918, observed an unusually severe outbreak. Chou (20) notes *Pleospora graminea* Died. on barley among the pathogenic fungi prevalent in the locality of Nanking in China. In India, the stripe disease has been studied by Butler (19), who states that the damage caused in that country is less than that recorded in European literature.

The symptoms of the stripe disease were described correctly by von Post (111), Pammel (102), and Rostrup (124), and in an especially detailed manner by Ravn (115). The first evidence of infection may often be recognized in the unfolded leaves of young seedlings by the presence of small pale spots, although generally at this stage the manifestations of abnormality are not pronounced. Later, usually about 6 weeks after sowing, after a number of leaves have become fully developed, these organs begin to show longitudinal, etiolated, yellowish stripes, often extending from the base of the blade to the tip. A single broad blade may show from 5 to 7 of these stripes although a smaller number is much more common. Not infrequently the stripes are interrupted and are thus replaced by numbers of elongated yellowish streaks. In any case, the similarity to ribbon grass then constituting one of the most striking characteristics of the disease, is soon modified by the appearance of dark brown discoloration in longitudinal elongated streaks. These brown discolored streaks are most apt to occur especially at the margins of the yellowish stripes, the tissues of which have now become dry and brittle, but often extend also as brown lines beyond the base of the blade, into the upper portions of the sheath. Often the brown streaks are separated from the remaining healthy parts by a yellowish zone within which the chlorophyll has largely disappeared. At this intermediate stage, especially when the brown streaks are short and relatively wide, the disease is apt to be mistaken for netblotch or spotblotch. Later the likelihood of a wrong identification is again diminished, for finally the remaining green tissues are also involved, the yellow and brown discolorations gradually fade into a uniform dark gray or brownish gray, the dead, rather brittle tissues split freely, and a dark efflorescence consisting of the abundant fructification of the parasite makes its appearance on the longitudinal foliar strips. (Pl. 1, A.) As the mechanical rigidity of the diseased leaf tissue becomes greatly lessened during the later stages, the leaf blades or their shredded remains (Pl. 1, Ab-e) usually droop or hang down in a characteristic way.

The effect of the parasite on the stem is equally pronounced. The elongation of this organ incident to heading, especially of the uppermost internodes, is considerably reduced or sometimes almost entirely suppressed. As a result the height of the plants is correspondingly reduced, diseased specimens generally not attaining much more than half the height of healthy individuals. Most frequently the spike never becomes visible, but remains entirely enclosed in the basal portion of the upper leaf sheath. In other instances, the inflorescence is thrust into the upper part of the upper leaf sheath, and, failing to emerge normally, protrudes partly from the opening on the side of the latter. (Pl. 1, A.) This abnormal mode of emergence generally involves some distortion of the awns, as

these tend to remain wedged in the sheath, from which, indeed, they may fail to become completely liberated. The condition thus brought about is in a measure characteristic of the disease, although Hegyi (57) reports a similar type of malformation due to the attack of aphids and to a period of cold weather at a critical time just before heading. In a relatively small proportion of plants, the inflorescence emerges from the sheaths, but for a smaller distance than in normal plants. However, even then the ovaries rarely develop anything beyond abortive seeds, the enveloping lemmas and paleas showing a decided brownish tinge. Viable seed certainly is not generally produced, the few instances recorded by Ravn being apparently more or less exceptional.

In southern Wisconsin, the life of diseased plants usually comes to an end between June 20 and June 25, depending to a large extent on the earliness or lateness of the season. Although at first readily distinguishable in the field, the dead plants are soon hidden as a result of the continued growth of the healthy individuals. By harvesting time, ordinarily soon after the middle of July, they have usually collapsed to such an extent that their remains are not found without special search. Owing to the production of immense numbers of fructifications during the several intervening weeks, particularly on the dead foliar parts, the spores of the fungus may be distributed to the floral parts of healthy plants. The experimental work of Ravn showed that the reappearance of the disease in successive seasons is due to the resultant contamination or infection of the seed with spores of the fungus. When seed thus naturally inoculated germinates, the seedling tissues are immediately infected. As the growth of the fungus keeps pace with that of the host, the parasite maintains itself in the growing point and, indeed, in all parts of the plant, even when at the time no outward symptoms are visible. The development of the disease, including its eventual manifestation toward flowering time, thus presents a striking analogy to that of the systemic smut diseases, and is brought about by a similar mode of parasitism of the fungus concerned. To this manner of development also must be attributed the fact observed by von Post (111), Rostrup (124), Pammel (102), Ravn (115), and others that the affected single individual plants or affected stools are quite uniformly diseased in all their parts, and are, with possible rare exceptions, totally destroyed. The use of terms like "moderate," "slight," "more or less," while not inappropriate in describing the extent to which a barley crop may be affected by stripe, is altogether inapplicable in describing the severity of attack of individual plants, and can in such connection be interpreted only as evidence indicating an erroneous diagnosis.

While in morphology *Helminthosporium gramineum* exhibits no striking characteristics, the writer is of the impression that its similarity to other congeneric species, particularly to those occurring on the same host, as well as on other cereals, seems to have been somewhat exaggerated. It is certainly conspicuously different from *H. sativum*, and is usually not so difficult to distinguish by microscopical examination of its fructifications from *H. teres* and *H. avenae* as Ravn's statements might lead one to believe. The sporophores occur usually in clusters of 2 to 6, fascicles of 3 to 5 being common (Pl. 1, Ea-Ee), whereas the corresponding clusters of *H. teres* (Pl. 2, Ea-Ec) and *H. avenae* (Pl. 4, De-Dg) rarely include a larger number than 3. Although, just as in the latter two species, the basal segment is considerably distended, the width of the sporophores of *H. gramineum* is perceptibly smaller in the

distal portion, measuring usually approximately  $6\ \mu$  as compared with  $7$  to  $9\ \mu$  for those of *H. teres* and *H. avenae*.

The spores vary from subhyaline when newly proliferated to yellowish brown when fully mature, never, however, becoming dark olivaceous, like those of *H. sativum*. They are (Pl. 1, Ba-h) typically straight or very slightly curved; subcylindrical, but quite frequently widest in the basal portion and tapering more or less toward the apex. Both apical and basal ends are rounded off, abruptly presenting a hemispherical contour. The peripheral wall is relatively thin as in other species having subhyaline or light-colored spores. The septa vary from 1 to 7 in all the material the writer has had occasion to examine, and are only infrequently associated with perceptible constrictions (Pl. 1, Bf, h), while in the spores of *H. teres* (Pl. 2, Ca) constrictions are more common and often moderately pronounced. Germination takes place promptly when the spores are mounted in water, usually within 30 minutes. Germ tubes are proliferated usually from both end segments and from several intermediate segments, the numbers produced from the basal segment varying from 1 to 3, while the other segments rarely give rise to more than one (Pl. 1, Ca-d).

A phenomenon undoubtedly related to germination is the production in nature of secondary spores or short secondary fructifications from primary spores, that in this species occurs to a greater extent than in the other congeneric forms parasitic on cereals, and which suggests comparison, for example, to *H. catenarium*. The secondary spores are not generally produced directly on the apex, but on a sporophoric process arising from the apex (Pl. 1, Dc, e) or obliquely from the apical cell (Pl. 1, Da, d) or less frequently laterally from the basal segment (Pl. 1, De). Not infrequently the process remains short and gives rise to only a single spore (Pl. 1, De), while again it grows out into a sporophore bearing half a dozen spores (Pl. 1, Dc). Evidently such secondary development may take place after the primary spore has become detached from the sporophore on which it was borne, as it usually is associated with a collapse of some or all segments of the primary spore involved (Pl. 1, Da). The secondary spores (Pl. 1, Dca-cc, Dda) may be nonseptate or show 1 to 3 cross walls; they are, in general, decidedly smaller than the primary ones, the minimum dimensions approximating  $11$  by  $20\ \mu$  (Pl. 1, Dca-cc, Dda). As the smaller individuals of the one order can not be readily distinguished from the larger individual of the other, all gradations may be found between these magnitudes and the maximum dimensions of the primary spores,  $20\ \mu$  for width,  $105\ \mu$  for length. The maximum measurement for spore length of *H. gramineum* is thus barely two-thirds as large as that of *H. teres*—a fact of great importance in identifying the species by microscopical examination.

Diedicke (29), in 1903, reported from Germany the discovery of an ascigerous stage of *Helminthosporium gramineum* in a species of *Pleospora*, which he found to correspond fairly well to *P. trichostoma* (Fr.) Winter. However, as the latter appeared to include a number of forms parasitic on different hosts but not interchangeable in their host relations, he regarded it as a collective species, and separated it first into a number of biologic races (28) which later he recognized as individual species (29). The perithecial form collected on old barley straw in a field that had been affected severely with the stripe disease was accordingly referred to a newly derived species, *P. graminea*. On inoculating barley leaves with sclerotia of the fungus, typical symptoms of stripe

are reported to have appeared in a number of cases, thus presumably establishing its identity. Diedicke's findings were confirmed by Noack (95) who, working independently, studied apparently the same ascigerous fungus collected on barley stubble in Hesse, Germany. Using conidia, ascospores, and sporulating material of host leaves, Noack secured infection by inoculating the first leaf of barley seedlings when it had attained a height of only several centimeters; and also on plants about 4 weeks older. The symptoms induced in the young seedlings were systemic, whereas on the older plants local infections manifested by the appearance of brownish spots were obtained.

The work of both Diedicke and Noack is open to the criticism that it was not done in connection with parallel infection experiments carried out with *Helminthosporium teres*, and that consequently these authors were, perhaps, not so capable of distinguishing between the pathological effects of the two related fungi as might have been desired. It may be mentioned that their fungus was obviously very similar to the form discussed in this paper as *Pyrenophora teres* (Died.) collected by the writer near Madison, Wis., where it occurred in abundance in spring on barley stubble apparently regardless as to whether or not the crop of the preceding year had been seriously affected with stripe. Recently, Paxton (106) has reported the occurrence of the mature perithecial stage of *H. gramineum* on two-year-old barley straw in California. Transfers of ascospores to corn-meal agar resulted in the production of conidia, which when used to inoculate barley gave rise to typical stripe lesions. According to Van Poeteren (109), perithecia of the parasite have been found on the glumes of germinated grains of barley, the seedlings of which exhibited the symptoms of stripe. This author suggests incubating seed in a moist warm atmosphere for three days in order to determine in advance from the appearance or nonappearance of perithecia whether it will give rise to diseased or healthy plants.

Of all the diseases due to species of *Helminthosporium*, the stripe disease undoubtedly has been made the subject of the largest amount of investigation aiming at effective methods of control. As the effective inoculum is presumably very largely, if not entirely, borne on the seed, the disinfection of the latter by the use of various fungicides and by the application of heat has not been without a considerable measure of success. Copper sulphate was found effective by Riehm (119) when applied in 1 or 0.5 per cent solution for 30 minutes; by Lind and Ravn (81) when applied in 0.5 per cent solution for 4 hours; and by Müller and Molz (93) when applied in the same solution for 16 hours. Lind and Ravn (81) obtained satisfactory results by the application of formaldehyde in 0.2 per cent solution for 6 hours; Schander (129) in 0.2 per cent solution for 30 minutes; and Johnson (70) in 0.16 per cent solution for 3 hours at 20° C., in 0.25 per cent solution for 2 hours at 10°, and 0.25 per cent solution for 1 hour at 25°. According to Lind and Ravn (81) treatment with mercuric bichloride in 0.1 per cent solution for 1 to 2 hours gives good results. Riehm (119) found 0.2 per cent mercury chlorphenol applied for 15 minutes effective. The hot-water treatment of seed for the control of stripe was found by Lind and Ravn (81) less effective than chemicals and of value only for lightly infected seed; Johnson (68), however, found a modified treatment with hot water 5 hours cold, and 15 minutes at 52° C., quite valuable. A variety of intermittent hot-water treatments have been devised, consisting in dipping the seed in hot water for relatively brief periods alternating with



longer periods of exposure to cooler water. Müller and Molz (93), as well as Lind and Ravn (81), found hot-air treatment worse than useless for stripe, as it tended to increase the proportion of diseased plants; Atanasoff and Johnson (3), on the other hand, found that such treatment markedly reduced the disease without materially injuring the seed. As the disinfection of barley seed usually involves a number of diseases, the choice of any particular treatment often is contingent on its effectiveness in controlling smut, netblotch, spotblotch, and the bacterial blight as well as stripe.

Owing to the successful control of stripe by seed treatment, not much attention has been devoted to other methods of combating the disease. The various reports already mentioned, of the occurrence of an ascigerous stage, and the statement by Paxton that 16-year old herbarium specimens of cultivated barley affected with *Helminthosporium gramineum*, when placed in a moist chamber, produced conidiophores and viable conidia from the dormant mycelium, suggest the possible value of measures involving sanitation. Indeed, Frank (43), Weiss (157), and later Jacevski (66) recommended rotation of crops, though perhaps more on general principles than because of possible experimental evidence or knowledge of the presence in fields of an overwintering stage playing an important part in establishing the parasite on successive crops. Ferraris (40) recommended burning the stubble in addition to seed disinfection. The removal and destruction of diseased plants found effective in Scotland (134) would obviously constitute a method of eradication too laborious to be seriously considered in the United States. The observations of various investigators, notably of Hori (63) and of Lind and Ravn (81), show that the proportion of diseased plants is increased when the seed germinates in soil at a low temperature. Müller and Molz (93), however, did not find late sowing in a warmer seedbed advisable; for, although less disease developed, the delay brought about a decrease in the yield.

Von Post (111) observed considerable differences in susceptibility to stripe between varieties of barley. Ravn (115) found the six-rowed varieties as well as the *erectum* types more heavily attacked, in general, by *Helminthosporium gramineum* than the *nutans* types, a condition exactly the opposite of that he found to obtain with reference to attack by *H. teres*. Kiessling (76), while unable to confirm Ravn's findings regarding the comparative susceptibility of the nodding and the erect types, noted pronounced differences in the proportions of diseased plants present in the stands of different varieties. It is worthy of note that some of Kiessling's strains, representing genetically pure lines, manifested with fair consistency moderate susceptibility, while other pure lines exhibited a high degree of resistance. This author urged, very justifiably, that the plant breeder ascertain the measure of resistance to stripe inherent in any varieties of barley with which he may be dealing and reject those lines showing marked susceptibility.

#### HELMINTHOSPORIUM TERES SACC.—PYRENOPHORA TERES (DIEDICKE)

*Helminthosporium hordei* Eidam 1891, in *Der Landwirt*, Bd. 27, p. 509.

*Pleospora teres* Died. 1903, in *Centralbl. f. Bakt. Abt. 2*, Bd. 2, no. 2, p. 52-59.

The binomial *Helminthosporium teres* was applied by Saccardo (126, pl. 833) to a fungus collected on leaves of barley near Padua, March, 1881, and figured in the "*Fungi Italici*" as having 3-septate conidio-

phores arising in a group of five from a green substratum and bearing at the tip a single conidium. The latter were represented as dark green structures, thick walled, 4 to 5 septate, ellipsoidal or subcylindrical, tapering perceptibly toward the rounded ends. A part of a leaf, showing a green region interspersed with uniform brown elliptical areas marked with short, black, longitudinal lines, was doubtless intended to represent the pathological habit of the fungus. In 1882 (127), a brief diagnosis of the species was published:

● *Maculis oblongis amphigenis, olivascentibus; hyphis fasciculatis, 100-130=12, cylindraceis basi subincrassatis, fuligineis; conidiis acrogenis cylindraceis, rectis utrinque rotundatis, 110-115=18, 4-5 septatis, non constrictis, obscure olivaceis.*

Neither the figures nor the text make it possible to identify definitely Saccardo's fungus with any one of the three species of *Helminthosporium* parasitic on barley. The number of sporophores in a fascicle, and the number of septa in the spore, suggest the form causing stripe; the thick wall, tapering ends, color, and absence of constrictions in the contour of the spore, suggest that causing spot-blotch; while the excessive width of the sporophore and the relative straightness of the spores suggest that responsible for net blotch. Although size of spores and appearance of lesions clearly point away from the stripe fungus, the former is nearly equally applicable to the other two species, while the latter is scarcely characteristic of any.

Nearly a decade later Briosi and Cavara<sup>5</sup>, in an account of the fungus causing leaf spot of oats, recognized it as a form of Saccardo's species. Oudemans (100), after examining Rabenhorst's specimens of *Helminthosporium gramineum*, concluded that the latter fungus was identical both with the one redescribed by Eriksson (37) under the same name and with *H. teres* Sacc. Ravn (115), however, did not accept Oudemans' views in their entirety, but in distinguishing two diseases of barley applied Rabenhorst's binomial to the fungus causing stripe, and Saccardo's to the causal organism of barley "Helminthosporiosis" or net blotch. In making these dispositions he took into consideration the destructive character of the parasite described by Rabenhorst in contrast to the local foliar lesions which, while figured and described very poorly by Saccardo, he was nevertheless able to identify with those characteristics of "Helminthosporiosis" by an examination of Saccardo's original specimens. The Italian mycologist, moreover, confirmed Ravn's opinion that his *H. teres* was the species responsible for "Helminthosporiosis."

The disease itself had not escaped earlier observation by other students. As was pointed out in another connection, Eriksson's specimens of *Helminthosporium gramineum* Rab. showed typical lesions of net blotch as well as of stripe; and his note on the "bladfläckensjukdom" indicates that the former was moderately abundant in the field. Kirchner (77), in 1891, had published an account of the "brown-spottedness" (Braunfleckigkeit) of barley leaves, observed in southern Germany during the preceding two seasons. The spots were described as blackish brown in color, narrow, often over 1 cm. in length, and surrounded by a narrow yellow zone while the leaf is still green. With the multiplication of the spots the leaves were observed to wither and give rise to the fructifications of the fungus. Material was submitted to Eriksson, who pronounced the fungus identical with that distributed by him, although

<sup>5</sup> BRIOSI, G. et J. CAVARA. I FUNGI PARASSITI DELLE PIANTE COLTIVATE OD UTILI. No. 80, Pavia, Italy. 1889.

Kirchner called attention to the fact that up to eight septa were present in the spores. This fact, together with the relatively mild nature of the disease, indicates that he was most probably dealing with net blotch. In 1898 Rostrup (125) had discussed a disease of barley which he designated as a "leaf-spot disease" (*Bladplelsyge*), characterized by the presence, largely on the lower, less vigorous leaves, of elongated dark brown spots, surrounded by a narrow yellow margin. This malady, which was not observed to be very destructive, Rostrup attributed to *H. gramineum* Rab. Apparently the same trouble had been investigated later also in Silesia by Eidam (35), who attributed it to a new species of *Helminthosporium*, *H. hordei*, which he found was not transmissible to oats and consequently different from the congeneric parasite causing leaf spot of the latter host.

The disease is evidently widely distributed in the United States, the records of the Plant Disease Survey containing reports of its occurrence in 21 States, including all the barley-growing districts of any importance. In this connection it may be mentioned, however, that although often responsible for appreciable loss, net blotch is of relatively minor economic importance compared to stripe or spot blotch. It is true that in Bakke's paper (6), which contains the first published record of the disease in this country, *Helminthosporium teres* is represented as probably the most serious parasite of barley. As Bakke failed to distinguish between the disease under consideration and the much more serious trouble attributable to *H. sativum*, the exaggerated account of the destructiveness, as well as the somewhat inaccurate morphological treatment of *H. teres*, both of which unfortunately appear to have been incorporated in Butler's handbook (19), are readily explained.

The course of development of netblotch has been described in detail by Ravn (115). In the vicinity of Madison, Wis., where in 1919 the writer had occasion to observe the progress of the disease, the regular crops exhibited the characteristic symptoms in small measure during the earlier part of the season. Later the lesions sparingly present on scattered leaves were completely obliterated by the blotches due to *Helminthosporium sativum*, that began to appear in great profusion at the heading stage about June 20. A much more favorable opportunity to study the disease presented itself with its development on volunteer plants from the latter part of August until the beginning of November, when netblotch was present in considerable abundance, to the practical exclusion of both stripe and spotblotch. The writer is informed that a somewhat suppressed manifestation of the trouble on the regular crop, and its extensive distribution on the volunteer crop, is, in general, not altogether uncharacteristic of its seasonal occurrence in our northern latitudes.

According to experiments reported by Ravn (115), the disease is propagated by seed infected or contaminated by the fungus. When such seed germinates at a relatively low temperature, as, for example, 10° to 15° C. or less, there results a local infection of the first seedling leaf which he designated as primary *Helminthosporiosis*. This is in contradistinction to secondary *Helminthosporiosis* resulting from infection by conidia developed on the dead tissues involved in the primary or in subsequently developed secondary lesions. On the other hand, when germination took place at a temperature of 20° C. or above, primary *Helminthosporiosis* failed to develop even though the seed was infected or contaminated. That such thermal relations are effective

in the development of primary lesions in the field was shown by the virtual absence of such lesions from the first leaf of plants resulting from sowings made in July and the first half of August, in contrast to a high percentage of infection secured from sowings made in March and April. The prevalence of netblotch on volunteer barley in the northern latitudes of our Middle Western States, where the temperature in midsummer ordinarily is at least as high as in Denmark, is accordingly on a *priori* grounds not to be attributed to seed infection. And, indeed, the abundance of the lesions on the first few leaves of a large proportion of volunteer seedlings in the fields kept under observation by the writer in 1919 indicated secondary infection due to spores from stubble and other remains of the regular crop.

Barley leaves affected with netblotch usually are not difficult to distinguish from leaves attacked by stripe or spotblotch. The presence of the parasite in the living foliar tissues is manifested by the appearance of dark brown spots or streaks which at first may be barely perceptible, measuring perhaps not above 1 mm. in length, but later increasing considerably in size, although not frequently measuring more than 20 to 25 mm. in a longitudinal direction. The increase in width usually is relatively small. A variegated appearance simulating that of the foliage of ribbon grass, due to alternately placed green and yellow stripes, is never produced, although the brown streaks characteristic of the later intermediate stages of stripe may sometimes be approximated in leaves affected by *Helminthosporium teres*. The most distinctive feature of the discolored areas involved in netblotch lesions is to be found, however, in the irregular distribution of the brown pigment, the latter being accentuated in very narrow lines, some oriented longitudinally, others transversely or obliquely to the axis of the leaf. As a result, a more or less irregular dark brown reticulate pattern may be distinguished within the areas of diffused brown. (Pl. 2, A, B.) Indeed, the pigmentation of the interstices within the reticulate pattern may become so reduced that the discoloration is present almost exclusively as a sharply defined network of brown lines. (Pl. 2, B.) The resulting macroscopic appearance, utterly different from that characteristic of stripe or spotblotch, and duplicated (as far as the writer is aware) only in leaves of meadow fescue affected by *H. dictyoides*, led Johnson (3) to apply to the disease the descriptive name "netblotch," which has since been generally adopted in the United States. The parts of the leaf immediately adjacent to the discolored tissue usually shows more or less etiolation that becomes manifest in the appearance of a narrow yellowish zone surrounding the spots. (Pl. 2, B.) Sometimes, especially in incipient lesions in which the reticulate character is pronounced, the measure of etiolation is apparently small, and the dark brown netlike lines are found in leaf tissue apparently very little changed. Later, however, all the lesions show yellow margins, which eventually become extended until the whole leaf blade is involved and withers from the tip to the base. (Pl. 2, Ad, Ae.) At this stage the spots, which may number several score on a single leaf and often become more or less confluent, begin to fade from a dark brown to a more diffused dull brownish gray. Not much later the fructifications appear as a light efflorescence extending usually from the brownish spots over the surrounding yellowish gray portions of the dead foliar organ.

The injury due to *Helminthosporium teres*, like that occasioned by most of its foliicolous congeners is thus the result of the destruction of

leaf tissue. As long as weather conditions are suitable, each successive leaf to be unfolded sooner or later becomes infected and in time may be killed. Plate 2, A, showing a volunteer seedling with five leaves, the first (Pl. 2, Ae) entirely withered, the next three (Pl. 2, Aa-b) exhibiting increasingly earlier stages, and the fifth (Pl. 2, Aa) apparently healthy, drawn from material collected September 2, 1920, represents a rather severely affected specimen. It may be mentioned that after September 15 the production of new lesions was much slower, with the result that, on November 3, the upper leaves of the plants, then about 24 inches tall, were entirely free from the disease, although fresh lesions could be found on the leaves lower down, while the lowermost withered foliage was covered with an abundance of profusely sporulating conidial fructifications.

The brown or olivaceous conidiophores (Pl. 2, Ea-d), emerging from the stomata or between epidermal cells, singly or in groups of 2 or 3, vary usually from 120 to 200  $\mu$  in length, and above the swollen basal cell, from 7 to 9  $\mu$  in diameter. They are thus somewhat stouter than those of *Helminthosporium gramineum* and *H. sativum*, besides being less closely septate, the septa occurring at intervals varying usually from 15 to 60  $\mu$ , and averaging approximately 35  $\mu$ . The writer has not observed them in fascicles of 5 and 6, indicating that their occurrence in groups of such number is at least less common than in the case of the stripe fungus. On the other hand, the conidiophores of *H. teres* appear to be quite indistinguishable from those of *H. avenae* in all respects.

Although *Helminthosporium teres* has been confused with both *H. gramineum* and *H. sativum*, the spores of the netblotch fungus are certainly not readily mistaken for those of the latter species. The writer's material, collected near Madison, Wis., October 28, 1919 (after a protracted period of damp, cloudy weather under conditions apparently nearly optimum for sporulation) showed these structures (Pl. 2, Ca-f) to vary from 30 to 175  $\mu$  in length and from 15 to 22  $\mu$  in width. In respect to spore length, therefore, the species is altogether superior to the two congeneric forms occurring on barley and inferior to *H. bromi* in approximately the same degree as the latter is inferior to *H. giganteum*. From 1 to 10 septa were found present, the septa, after the delimited segments have partly rounded up, being associated usually with perceptible constrictions in the contour of the spore. As the constriction at the proximal septum is especially pronounced and constant, and the basal, as well as the apical end, is rounded off in a hemispherical form, the basal segment is given a subglobose shape as characteristic for the species as are the basal segments of *H. tritici-repentis* and *H. bromi*.

The spores are subhyaline in color, like those of the latter two species, when newly proliferated, but become greenish fuliginous or yellowish when older, usually, however, not assuming a tinge quite as dark as the brownish yellow of fully matured spores of the stripe fungus, and consequently never approximating the dark olivaceous color of those of *H. sativum*. Associated with this light color is a thin peripheral wall, the drawings of Saccardo (126) and some other authors showing the outer wall as a thick structure being apparently based on dead material. While the spores of *H. gramineum* usually are entirely straight, those of the netblotch fungus not infrequently exhibit slight irregular crooks (Pl. 2, Cf), in which respect as well as in the general subcylindrical shape and location of the hilum within the contour of the basal end, a similarity to *H. bromi* and *H. tritici-repentis* again is evident.

When the spores are mounted in water, they germinate promptly, germ tubes being produced laterally or obliquely from intermediate as well as end segments, the protoplasm changing from a uniform to a regularly vacuolate structure. (Pl. 2, Da-d.) Like those of related species they are relatively short lived, a considerable proportion of the segments dying during the first 10 to 15 days of exposure, and probably few surviving after two months. It appears very probable that Bakke's account (7) of the longevity of *Helminthosporium teres* was based on material not of this species, but of *H. sativum*.

On media containing little organic food material, like tap-water agar (tap water 1,000 cc. agar-agar 20 gm.), or Beyerinck's agar (distilled water 1,000 cc., ammonium nitrate 0.5 gm., dipotassium phosphate 0.2 gm., magnesium sulphate 0.2 gm., calcium chloride 0.1 gm., ferrous sulphate trace, agar-agar 15 gm.), aerial growth, although sparse, often consists almost entirely of conidial fructifications bearing spores similar in structure and color to those developed in nature, but usually much shorter and containing only 2 to 3 septa. A strong tendency toward production of secondary spores is apparent also in artificial cultures, giving rise to conditions like those illustrated by Ravn (115).

On media containing a large amount of organic food material, a profuse white aerial growth results, consisting partly of fluffy mycelium, and partly of more or less compact erect columnar masses. The submerged mycelium shows abundant anastomosis with the formation of numerous complexes composed of dark brown inflated or lobulate segments. (Pl. 9, Fa-b.) The latter apparently represent incipient stages of sclerotia which on sterilized barley straw may become readily visible to the naked eye, often exceeding 0.5 mm. in diameter. Ravn, although failing to observe any indication of ascus formation in these cultivated bodies, nevertheless interpreted them as immature perithecia of a Pyrenomycete, probably related to *Pleospora polytricha* (Wall.). This interpretation was justified by Johnson's (69) discovery in Wisconsin of the ascigerous stage of the netblotch parasite in a fungus he referred to the genus *Pleospora*, the specific identity of the stages in the pleomorphic forms being supported by apparently conclusive inoculation and cultural studies. The same ascigerous form was collected by the writer on barley straw and stubble from fields near Madison, Wis., late in March, 1919, as well as in March and April of the following year. As Diedicke (29) and Noack (95) recognized a similar ascigerous form as the perfect stage of *H. gramineum* in Germany, it may not be superfluous to mention that the Pyrenophora fructifications found on barley stubble in Wisconsin do not seem to be confined to weak culms such as might conceivably represent the remains of plants affected with stripe, but are found very abundantly on culms which, because of their manifest normal size and attachment to a perfectly developed head, can not easily be supposed to be derived from "striped" plants. (Pl. 3, A.) Previous to the publication of Noack's paper, Diedicke (29) had suggested the binomial *Pleospora teres* for the ascigerous stage of *H. teres*, at that time unknown, and consequently somewhat hypothetical.

The perithecia on barley straw (Pl. 3, B) are of the same type as those of *Helminthosporium bromi* and *H. tritici-repentis*. In general, however, they are perceptibly larger, measuring usually about 0.5 mm. in diameter. Although the lateral wall may sometimes taper toward the top, a distinct ostiolar beak usually is not present, and in most instances the ostiole is represented by a mere opening in the apical portion of the fruiting body. Setae may be altogether absent, or present in moderate number not

usually near the ostiole but on the lower portion of the wall. They (Pl. 3, Ca) differ from the sporophores (Pl. 3, Cb, Cc) usually produced in large numbers on the upper surface of the fruiting body in spring, in being dark olivaceous rather than of a brown color, in being more closely septate, and in tapering toward the tip to about one-half their basal diameter. Although material of *Pyrenophora tritici-repentis* and *P. bromi* collected near Madison, Wis., in the spring of 1919 and 1920, showed asci and ascospores in excellent condition, the development of corresponding structures in *P. teres* did not go beyond a more or less abortive stage. In most instances the asci remained small; if ascospores were delimited, they usually failed to grow to normal dimensions, and frequently showed no cross walls. Plate 3, D represents approximately the least abnormal conditions found in the season of 1920, showing each of the asci with several obviously normal spores, the remainder being either more or less misshapen, or having one or several or all of the segments collapsed. The space in the interior of the imperfectly developed fruiting body not occupied by asci, is filled with colorless vertically oriented, more or less filamentous pseudoparenchyma. As has been indicated in another connection, the failure of the asci to develop normally may be attributed to the advent of weather conditions in the spring encouraging the production of the numerous conidiophores (Pl. 3, B), the initiation of which appears to involve a cessation in the development of the internal parts of the perithecium. As abundant moisture and a relatively high temperature appear to favor the conidial stage, it is not illogical to expect that a long protracted period of cold, dry weather in spring might result in the production of more nearly normal asci.

Judging from the more satisfactory material examined, the asci of *Pyrenophora teres* are subcylindrical throughout most of their length, the proximal portion tapering toward the short stipitate base, and the wall of the apical end modified by a ring-like thickening. They measure 30 to 36 by 220 to 250  $\mu$ , and contain 8 spores in distichous arrangement. Normal specimens of the latter are light brown in color; measure approximately 18 to 22 by 52 to 60  $\mu$ ; and show three transverse septa associated with perceptible and often pronounced constrictions. One or both middle segments may, in addition, be divided by a longitudinal wall. The protoplasmic contents appear more or less granular and vacuolate. Germination takes place promptly when the spores are mounted in water, in a manner entirely similar to that of the two related ascigerous forms discussed in this paper.

It is difficult to estimate the measure of importance to be attributed to the ascospores and conidia developed by the perithecia in reestablishing the fungus at the beginning of successive seasons. The abundance of these fructifications, however, indicates that they constitute a source of inoculum that ought not to be overlooked, and certainly provides an additional reason for crop rotation, or such sanitary measures as turning under or otherwise disposing of the stubble of the preceding season. Most of the investigations relating to the control of netblotch have been carried out as subsidiary to control studies of the more destructive diseases affecting barley, particularly stripe, loose and covered smuts (*Ustilago nuda* and *U. hordei*); and spotblotch. In general, the seed treatments effective against stripe and spotblotch have also been found to reduce materially the number of primary infections of netblotch. Nevertheless the literature does not seem to indicate any treatment for the disease quite equalling in efficacy, for example, the various formalde-

hyde treatments that have been devised against stripe, or the hot-air treatment described by Atanasoff and Johnson (3) against spotblotch. Moreover, as the plant is subject to attack at any stage of growth, and the fungus can spread from a small number of diseased seedlings to other plants, reduction of primary lesions by methods aiming at the disinfection of the seed is of relatively less value than in the case of diseases, the occurrence of which is contingent on seed contamination and infection during the germination period.

#### HELMINTHOSPORIUM AVENAE EIDAM

*Helminthosporium teres* Sacc. forma *avenae-sativae* Briosi & Cavara 1889, in I funghi par. delle piante colt. od utili, no. 80.

*Helminthosporium avenae-sativae* (Br. & Cav.) Lindau in Rabenh. Krypt. Fl. V. Deutschland. Ed. 2, Bd. 1, Abt. 9, p. 34.

*Helminthosporium avenae* (Br. & Cav.) Ravn, 1900, in Bot. Tidsskr. v. 23, p. 212-213.

*Helminthosporium gramineum* of Ritzema Bos, Frank, Fraser not Rabenhorst.

In 1889 Briosi and Cavara<sup>6</sup> distributed specimens of a fungus collected near Pavia, Italy, where it was found parasitic on the leaves of oats, *Avena sativa* L., producing narrow, oblong, longitudinally elongated, olivaceous foliar spots, with dark margins. The infection was described as starting ordinarily at the tip of the leaf, where the first spots appear, and from whence the mycelium gradually invades the leaf parenchyma, until the entire blade withers and dies. The injury to the foliage thus occasioned was reported to interfere with the full development of the seeds. Briosi and Cavara designated the fungus itself as *Helminthosporium teres* Sacc. forma *avenae-sativae*, differing from the typical species by the greater length of the conidiophores, the occurrence of the latter singly instead of in fascicles, and the somewhat smaller dimensions of its spores. In the brief diagnosis of the form, accompanied by figures, the conidiophores are described as scattered, stout, cylindrical, many-septate, fuliginous, measuring 150 to 200 by 9 to 12  $\mu$ ; the spores as acrogenous, olivaceous, cylindrical, or slightly swollen in the middle, rounded at the ends, 4 to 6 septate, and measuring 80 to 110 by 15 to 16  $\mu$ . Their representation of the septa and spore wall as thick structures indicates that Briosi and Cavara used dead material for their studies, a circumstance to which may be attributed, perhaps, the inexact description of the color of the spore, and their failure to mention the more distinctive features of the fungus.

Eidam (35), in 1891, published an account of a leafspot of oats occurring in Silesia and affecting usually the first leaf, but sometimes also the second and third leaves of the host. He recognized the causal parasite as a new species, *Helminthosporium avenae*, distinct from his *H. hordei* (= *H. teres* Sacc.) because of the negative results obtained in his attempts to infect barley with the former, and oats with the latter. Ritzema Bos (121) later described an attack upon oats by *H. gramineum* Rab., the resulting foliar spots differing from those occurring on barley in being short, somewhat round, and associated with a reddish color of the diseased leaves. Ravn (115), as the result of comparative cultural and biometrical studies of the parasites causing stripe, netblotch of barley, and the "Helminthosporiosis" of oats, concluded that the latter represents an independent species.

It is of some slight nomenclatorial interest to note that Ravn, apparently in the belief that Briosi and Cavara's priority in recognizing the

<sup>6</sup> Op. cit., p. 657



parasite as a separate taxonomic entity necessitated the perpetuation of their form name as the specific name, adopted the combination *H. avenae* (Br. & Cav.). The last portion of the form name—*sativae*—he omitted purposely on the ground that it was unnecessary. Lindau (83, p. 34-35), presumably because of the patent irregularity in altering a name in such manner, recognized Briosi and Cavara's form name without alterations as the proper specific name, and consequently listed the fungus as *H. avenae-sativae* (Br. & Cav.). However, as the use of an earlier varietal name to replace a specific name (when the variety is raised to specific rank) is not sanctioned by present usage, it is obvious that both Ravn's and Lindau's combination are equally unauthorized, and that the proper combination is evidently the one established by Eidam.

In this connection, it may be mentioned that Cooke (23), in 1889, described as *Helminthosporium avenaceum* Curtis Herb., a fungus occurring on straw in the United States (oat straw according to Saccardo) the conidia of which were characterized as cylindrical to subfusoid, pale honey-colored, and measuring 75 to 85 by 15  $\mu$ . Harkness (50) mentioned *H. avenaceum* Curt. as having been found on Avena, at San Francisco, in April. Atkinson (4) records having collected a fungus to which he applies the same name, in Mississippi, on June 26, 1891. It is not impossible that these authors were dealing with the fungus originally described from Italy, inasmuch as the brief diagnosis published by Cooke is not greatly at variance with that of Briosi and Cavara, and in purely morphological details nearly as satisfactory for the parasite causing leaf spot of oats as the latter. Since in none of the American writings was the fungus associated with any lesions in the living plant, such possible identity can not readily be established. The question is further complicated by the occurrence of forms of *Helminthosporium*, associated with sclerotia appearing saprophytically in considerable quantity on oat straw in spring, and evidently representing immature perithecia of *Pleospora* or *Pyrenophora*. The writer has investigated two such forms collected near Madison, Wis., in 1919 and 1920, one of which seems to be similar to or identical with *H. avenae*, while the other is entirely different, its small, dark olivaceous, strongly curved, 5-septate spores germinating by the production of 2 polar germ tubes. Further details may be published in a later paper.

In 1895, Harvey (51) published a brief account of a disease of oats found in Maine during the preceding two seasons, manifested apparently by premature yellowing of the foliage, and the subsequent production of dark brown spore masses that appeared as small dark dots or lines upon the affected leaves. The fungus in question was a species of *Helminthosporium*, which Ellis, to whom material was sent, identified as *Helminthosporium inconspicuum* C. & E. var. *britannicum* Grove. Unfortunately, however, as the only statement regarding the host relationship of Grove's variety in the diagnosis given by Saccardo (128, v. 4, p. 411-412) refers to fading grass leaves without any mention of species or genus, it is quite impossible, with the paucity of morphological detail, to identify it with any one of a considerable number of fungi.

Ellis's identification might be supposed to indicate that the Maine fungus corresponded in some measure with the diagnosis of the Warwickshire form:

Effusum bruneolum, hyphis subflexuosis, vix nodulosis, 4-5 septatis, pallide brunneis, 160-180= $\approx$ 10  $\mu$ ; conidiis elongis, diaphanis, endochromate brunneolo diviso, dein 3-5 septatis, 60-100= $\approx$ 18-22  $\mu$ .

That the correspondence is not especially close is indicated by Harvey's statement that the spores of his fungus were somewhat shorter, measuring 40 to 80 by 15  $\mu$ , and sometimes less frequently septate, namely, 1 to 5 times. And his figure of the spore, showing this body as an ellipsoidal, comparatively closely septate structure does not suggest any close resemblance either to *H. inconspicuum* C. & E. or to *H. teres*, between which Grove's variety was reported as occupying a median position. Nor is it any more suggestive of *H. avenae*. On the assumption that only one parasitic species of *Helminthosporium* occurs on cultivated oats, Harvey's fungus might perhaps nevertheless be supposed to be identical with the one distributed by Briosi and Cavara. An examination of specimens of diseased oats collected near Bloomington, Ill., in June, 1920, bearing conidiophores and conidia of a type somewhat different from that generally found characteristic of the oat leaf-spot fungus, has, however, made the writer inclined to believe that such an assumption might probably prove to be incorrect.

The disease caused by *Helminthosporium avenae* is widely distributed. It was reported early from Germany (35), Austria (56), Denmark (115), Belgium (88), Holland (121), and Italy. Fraser (45), in 1913, found the "stripe disease of barley" severely affecting oats in Quebec. Anderson (1, p. 105) reported *H. avenae* from Alaska. According to records of the Plant Disease Survey the *Helminthosporium* leaf spot of oats appears to have been observed in New York, Pennsylvania, Indiana, Wisconsin, Louisiana, Iowa, Minnesota, Nebraska, Montana, Idaho, and Washington. Undoubtedly, it occurs at least in all the northern States to a greater or less extent; the writer, for example, has found it quite abundant in Connecticut and Maine during the season of 1921, apparently wherever the host was cultivated. Yoshino (161) reported *H. avenae* as occurring in Japan, and Butler (19) records leaf spot as being very common in India, especially on young plants.

Ravn's investigations have shown that, in general development, the disease follows the same course as netblotch of barley. The infection of the seedling during the germination period results in the production of primary lesions on the first leaf; from the primary lesions the fungus spreads to the foliage, is disseminated later by successive generations of spores, and finally the maturing fruit is infected or contaminated to propagate the trouble the next season. The local symptoms of infection, on the other hand, however, are quite different. Instead of numerous spots exhibiting an irregular reticulate pattern of short accentuated lines or streaks, the affected oat leaves rarely show more than 3 or 4 brown spots. (Pl. 4, A.) It is true that the colored figure in Butler's manual shows a large number of lesions on a single leaf. Such a heavily spotted condition certainly has never been observed by the writer, and, perhaps, may be associated with a more severe manifestation of the disease in India.

✓ The spots may be broad and irregular, or long and narrow; in any case, the margins are frequently poorly defined, merging gradually into yellow, reddish, or orange shades which eventually spread over a large portion of the leaf blade. The gradual extension of diffused yellow and reddish discoloration appears to be coincident with the progress of the fungus in the affected tissue. In the absence, usually, of extensive brown conspicuously abnormal spots, the morbid decline of the leaf, due to the development of the parasite, simulates withering occasioned by weather conditions or maturation much more closely than in any of the three *Helminthosporio-*

rium diseases of barley. As a result, the damage to the oat crop due to leafspot, although undoubtedly not of major importance, is much more likely to be considerably underestimated than that caused by the majority of related diseases of other cereals. Thus, during the latter part of June, 1920, the oats observed by the writer growing in isolated patches in the eastern half of Long Island bore only meager evidence of being affected by leafspot. Nevertheless, four weeks later, microscopical examination revealed the fructifications and spores of *H. avenae* on more than half of the mature plants, sometimes in considerable quantity.

After the death of the affected leaf, the red and orange pigmentation very largely disappears, being replaced by a pale-yellow or gray color, and even the brown discoloration usually loses some of its intensity. At this point the conidiophores of the fungus make their appearance. As Ravn has pointed out, these structures (Pl. 4, Da-g) are very similar to those of *Helminthosporium teres* in all respects—color, dimensions, septation, and mode of emerging from the epidermis—although exhibiting sometimes a slightly greater tendency toward occasional branching (Pl. 4, Dc, De). The spores also resemble those of *H. teres* very closely, having approximately the same range in size and number of septa; and showing the same irregularly cylindrical shape, hemispherical ends, subhyaline to light fuliginous coloration, and mode of germination by the production from intermediate as well as end segments, of laterally or obliquely oriented germ tubes.

The writer was unable to confirm Ravn's finding that the conidia of *H. avenae* slightly exceed in length those of *H. teres*. In general, the dimensions of the two species appeared quite equal, and whatever slight inequality in length and width was observable was rather in favor of *H. teres*. It must be mentioned, however, that the fresh material used by the writer in the study of the two forms was not developed under comparable conditions, that of *H. teres* having been collected late in October during a damp, cool period seemingly especially favorable for sporulation; while the material of *H. avenae*, collected late in July, had manifestly developed at midsummer temperature. Perhaps the two fungi might better be regarded as biological forms of the same species, in the same sense in which such forms are recognized in *Puccinia graminis* and in the mildews. Whatever slight morphological differences, demonstrable in the conidial stage by biometrical methods, may exist between the two fungi, they could scarcely be of a larger magnitude proportionally than the differences between various biological forms of, for example, the stem rust fungus. A study of the ascigerous form of *H. avenae*, which, as has been suggested, the writer believes he has collected, although in very poor condition, ought to cast some light on its taxonomic relationship. For the present, it is advisable to follow Eidam and Ravn in regarding the parasite on oats as distinct from *H. teres*. Certainly the idea, proposed by Briosi and Cavara, of placing it as a morphological "forma" of *H. teres* is not tenable.

It may not be amiss to call attention to an error apparently caused by a partial misinterpretation of Ravn's paper, and more particularly of his widely copied but, perhaps, insufficiently representative figures of the conidia and conidiophores of *Helminthosporium gramineum*, *H. teres*, and *H. avenae*. In some general treatises, the impression is conveyed that the possible morphological difference between *H. avenae* and *H. teres* is approximately of the same order as the difference between *H.*

*teres* and *H. gramineum*. The differences between the latter two species are, in reality, altogether much larger. For example, while the largest spores of the fungi causing netblotch and leafspot of oats, seen by the writer, have measured between 170 and 175  $\mu$ , the longest spores of the stripe fungus have not been found to exceed 105  $\mu$  in length. The number of septa in the spore of the stripe fungus very rarely exceed 7, while in the other two species 8 and 9 cross walls may be found quite readily and even 10 or 11 occur in a small proportion of instances. Production of secondary spores, common in *H. gramineum*, is rare in *H. avenae* or *H. teres*. In short, *H. gramineum* is not closer to either *H. avenae* or *H. teres* than a moderate number of other congeneric species.

As leafspot usually is not a serious disease, and in the United States not very conspicuous in its manifestations, little attention has been paid to its control. Atanasoff and Johnson (3) have found the hot-air seed treatment effective in reducing the disease. The value of methods of control based on seed disinfection would appear to be contingent, at least to some extent, on the absence of a possible sclerotial or ascigerous stage, or the relative ineffectiveness of such a stage as a factor in the propagation of the fungus.

#### HELMINTHOSPORIUM TRITICI-REPENTIS DIEDICKE—PYRENOPHORA TRITICI-REPENTIS (DIED.)

*Helminthosporium gramineum* Rab. f. sp. *tritici-repentis* Diedicke olim 1902, in Centbl. Bakt.[etc.] Abt. 2, Bd. 9, p. 317-329.

*Pleospora tritici-repentis* Died. 1903, in Centbl. Bakt.[etc.] Abt. 2, Bd. 11, p. 52-59.

*Pleospora trichostoma* (Fr.) Wint. f. sp. *tritici-repentis* Noack 1905, in Ztschrif Pflanzenkr., v. 15, p. 193-205.

*Helminthosporium tritici-repentis* as recognized by Diedicke (28), first, as a biological or form species of *H. gramineum* Rab. and later (29), as an independent species, distinct from *H. teres*, *H. bromi*, *H. gramineum*, and *H. avenae*. It appears to be widely distributed; its perithecial form occurring very abundantly in our northern latitudes on the dead culms of quack grass, *Agropyron repens*. The visible effects resulting from the attack of the fungus on growing plants of quack grass are usually not at all conspicuous, any dark discoloration like that induced by the parasitism of *H. sativum* on the same host, being absent. The affected leaf gradually loses its green color and withers from the tip downward, changing at the same time first to a pale yellowish, and later to a gray color. As the foliage of the host developed after the earlier stages of growth is relatively rigid, the mechanical distortion associated with the death of any except the lower and more delicate leaves (Pl. 5, A) usually is not very noticeable.

According to Diedicke, the disease resembles barley stripe in affecting the whole plant, usually suppressing the development of the inflorescence, or preventing its emergence from the enveloping sheath. Although this opinion is not without some plausibility, anatomical evidence regarding the distribution of the fungus in the tissues of the plant would appear to be necessary before the disease can be regarded as systemic in the same sense as stripe. Some differences in the manifestations of the two diseases certainly are apparent. The symptoms do not become evident simultaneously in all the leaves of individual quack-grass plants, but are manifested first in the basal leaves, and later may appear in successively higher foliar organs. Nor do diseased leaves of *Agropyron repens* exhibit anything similar to the longitudinal variegation char-

acteristic of the foliage of barley infected with *H. gramineum*. The conidial fructifications of *H. tritici-repentis*, moreover, make their appearance scattered sparsely here and there over the surface of the dead leaves, not crowded on the affected parts like those of the stripe fungus. It is evident, therefore, that at least in some respects, the development of *H. tritici-repentis* on the host presents closer analogies to types like *H. dematioideum* and *H. siccans* than to *H. gramineum*.

The dark olivaceous usually 3 to 6 septate sporophores of *Helminthosporium tritici-repentis* (Pl. 5, Da-f) emerge from the stomata, or, more frequently, from between epidermal cells. Above the somewhat swollen basal segment, they vary in width from .7 to 9  $\mu$ , being thus perceptibly inferior in this dimension to the homologous structures of *H. bromi*. In length they vary from 80 to 220  $\mu$ . The conidia (Pl. 5, Ba-m) are typically subhyaline, straight-cylindrical, from 1 to 9 times septate, from 12 to 21  $\mu$  in diameter, and 45 to 175  $\mu$  in length. As in *H. teres*, the septa usually are associated with slight but perceptible constrictions in the contour of the thin peripheral spore wall.

The most distinctive peculiarity in the spores of this species is found in the shape of the basal segment, the proximal portion of which usually tapers abruptly in the manner of a cone to be rounded off or flattened near the hilum. In profile the basal segment thus is remotely suggestive of the horizontal aspects of the head of a snake, while the distal end usually is rounded off in a hemispherical form (Pl. 5, Ba, e, j, k). It may not be superfluous to add that departures from this type are not infrequent. The 2 or 3 terminal segments may taper appreciably toward the distal end (Pl. 5, Bb, c, d, m) or the width of the different segments may vary considerably (Pl. 5, Bg, m) or the axis of the spore may be somewhat curved (Pl. 5, Bc). Germination begins within an hour after the spores are mounted in water. Each segment is capable of producing a germ tube, the larger spores (Pl. 5, C) thus producing from 6 to 8 or more germ tubes, although usually one or several cells may remain seemingly inert.

The ascigerous stage of this species of *Helminthosporium*, as mentioned before, occurs in great abundance on the dead culms of *Agropyron repens* and to a small extent also on the leaves, particularly on the sheaths. (Pl. 6, Ba-b.) Although the black perithecia are readily discernible in autumn, their subsequent maturation appears to be rather slow. In the vicinity of Madison, Wis., mature perithecia have been collected from early in April until well toward the end of May. When developing in the looser tissue of the leaf, especially after some decay has taken place, the imbedded portion usually is distinctly subspherical, from 0.2 to 0.35 mm. in diameter, and tapers into a short, well-defined beak as shown in Plate 6, A. However, when developing in the harder tissue of the culm, the perithecium usually is much more irregular in shape and the beak less readily recognizable as a special modification. In any case, the beak usually bears near the ostiole a number of dark-brown sterile setae which may be straight or flexuous, continuous or several times septate, occasionally branched, and varying in number from several to over a score. As Diedicke pointed out, under certain conditions a large number of conidiophores and conidia may be produced from the upper side of the perithecium, the further development of the ascospores, if not complete, then often being checked altogether. This tendency toward conidial production usually is well expressed in the case of those perithecia found on the upright culms; on the other hand, when the host material is loosely

covered with leaves or other rubbish, it often is entirely suppressed. There can be little doubt that the environmental factors of temperature and moisture are of primary importance in this connection.

Diedicke recognized this perithecial form as a new species, *Pleospora tritici-repentis*, which he distinguished from *Pleospora bromi* largely because of the smaller dimensions of its perithecia, asci, and spores, and because of the failure of reciprocal inoculations to produce infection. Noack (95) did not accept the taxonomic dispositions made by Diedicke but regarded the perithecia on *Bromus asper* and *B. inermis* as not different morphologically from those on *Agropyron repens*, or from the perithecia on barley presumably associated with the stripe fungi. He consequently reduced the parasites on quack grass and on the two species of *Bromus* to biological forms of *Pleospora trichostoma* (Fr.) Wint., which, like Diedicke earlier, he identified as the ascigerous stage of *Helminthosporium gramineum*. The fungi under consideration were accorded a dubious status in the general works published subsequently, like those of Lindau (83) and of Stevens (139).

A comparison of material collected by the writer near Madison, Wis., in the spring of 1920, showed the general correctness of Diedicke's statements regarding the relative sizes of the perithecia and asci of *Pyrenophora tritici-repentis* and *Pyrenophora bromi*. As the diameter of the perithecia of the parasite on quack grass appears to vary from 0.20 to 0.35 mm. the inequality in size between the fruiting bodies of the two forms certainly is not pronounced. In *Pyrenophora tritici-repentis*, especially when developing on decaying leaves, the ostiolar beak usually is narrower, less massive, and consequently a somewhat more distinctive structure than in *Pyrenophora bromi*. The asci (Pl. 6, C) of *Pyrenophora tritici-repentis*, measuring usually from 170 to 215 by 43 to 50  $\mu$ , are similarly somewhat smaller than those of *Pyrenophora bromi*.

It may be mentioned that measurements of asci in fully matured living material are dependent to a considerable extent on the amount of moisture present, as these structures under dry conditions usually are found contracted tightly over the spores, while under moist conditions they swell until the space inside of the fruiting bodies is completely occupied. In any case, on being crushed out of the perithecia, they undergo very considerable swelling preliminary to the rupture of the ascus wall and the discharge of the eight ascospores. The latter, of a brownish color and measuring usually 18 to 28 by 45 to 70  $\mu$ , are uniformly three times transversely septate, often with one or both of the middle segments further divided by a longitudinal wall. The septa are associated with constrictions in the peripheral wall of the spore, which frequently is found enveloped in a gelatinous covering. (Pl. 6, Da, Db, Eb.)

Germination takes place promptly by the production of a germ tube from several or all segments. (Pl. 6, Da-b.) On media ordinarily employed in laboratories, like potato agar, a fairly compact mass of white aerial mycelium is produced, corresponding in all respects to the growth obtained by the use of conidia. Anastomosis with the resultant production of groups of inflated lobulate segments is abundant in the submerged mycelium, but large sclerotia or imperfect perithecia of a size readily visible to the naked eye, like those produced by *Helminthosporium bromi*, have never been observed in pure cultures of *H. tritici-repentis*.

The parasite on quack grass consequently is to be regarded not as a biological race of *Helminthosporium gramineum* but as an independent morphological species. While the ascigerous stage bears a strong resemblance to *Pyrenophora bromi*, as well as to a number of other congeneric forms found on graminaceous hosts, it is readily distinguishable at least from *Pyrenophora teres*. On the other hand the conidia which, like those of *H. teres* and *H. avenae*, show a range in length intermediate between the range of this dimension in *H. gramineum* and *H. bromi*, can not possibly be mistaken for the conidia of any of these species because of the characteristic contour of the basal segment.

#### HELMINTHOSPORIUM CATENARIUM, N. SP.

During the latter part of September, 1920, the writer kept under observation a stand of wood reed grass (*Cinna arundinacea* L.) near Brooklyn, N. Y., on the northern coast of Long Island. Although the season had not been a dry one, the grass, nevertheless, showed symptoms suggesting drought injury. The distal portions of most of the leaves had withered completely (Pl. 7, A) and, in some instances, the injury involved more than half of the blade. Less frequently, the foliar organs exhibited longitudinally elongated dry areas or spots within healthy green parts. No indication of any discoloration, either at the margins of the lesions or in the dried portions, was ever observed. As the disease presented many of the characteristics of white blast, quite common on sweet corn in the trucking district in the vicinity of New York City, some of the affected leaves were collected and examined in the laboratory. The microscope revealed an abundance of *Helminthosporium* fructifications on the affected leaves, particularly on those parts that had apparently been dead for some time.

As in other species of *Helminthosporium* occurring on graminaceous hosts possessing foliage with a relatively firm epidermis, the sporophores of the fungus on *Cinna arundinacea* are found to emerge very largely from the stomata. (Pl. 7, Ea-c.) Beyond being conspicuously thin walled, and rather unusually strongly geniculate at the points of attachment of the conidia, they exhibit no especially distinctive features.

The spores, which are colorless to light yellowish, however, show such a large measure of variability in shape and apparent development, that the fungus is easily recognized as one of the more aberrant and peculiar members of the genus. A considerable proportion of the spores are of moderate length, straight and tapering (Pl. 7, Ba, d, j), and perhaps could not be readily distinguished from those of *Helminthosporium dictyoides*, although the latter eventually become more deeply tinged with yellow. Usually only the shorter spores appear both straight and uniformly tapering. Generally, those in excess of  $80\ \mu$  are very perceptibly curved or bent in an irregular manner, and, in addition, the diameter of the different segments varies to a very considerable extent. The longer spores often show a decided median constriction. (Pl. 7, Bb, e.)

Frequently, a secondary spore (Pl. 7, Bi) is found attached at the tip of a primary one (Pl. 7, Bh), being characterized by smaller dimensions and, if immature, by the absence of septa. That the formation of secondary spores is not an unusual occurrence is indicated by the presence of a dark conspicuous scar at the tip of many spores, quite similar to the basal hilum and often associated with a peculiar prolongation of the distal

portion of the terminal segment, giving the spore a general contour crudely suggesting that of a rifle cartridge with contracted tip. (Pl. 7, Bc, f.) It thus will be seen that while the smaller spores resemble those of *H. dictyoides*, and in a measure those of *H. gramineum*, the larger ones are of a length not attained by the spores of any of these species, approximating most closely that of the spores of *H. tritici-repentis*. (Pl. 7, Bb.) The spores of the parasites on *C. arundinacea* and on *Agropyron repens*, moreover, show additional similarity in respect to coloration and general shape, both being subhyaline, as well as more or less irregularly curved and of variable width. The spores of *H. tritici-repentis*, however, have not been observed to show an apical hilum or apical attenuated prolongation; nor has the fungus on wood reed grass exhibited the peculiar modification of the basal segment characteristic of *H. tritici-repentis*.

In pure culture, on potato agar or corn-meal agar, the parasite on *Cinna arundinacea* grows readily, producing a white mycelium, both in the form of erect compact tufts 2 to 5 mm. in height and usually developed at the point of inoculation, or of minute superficial flecks scattered sparsely over the surface. In any case, the hyphae usually remain sterile for a number of days before the spores begin to develop terminally. After this stage is reached the sporophore, instead of developing by alternately elongating and proliferating spores, as is usual in the genus, usually develops in the manner somewhat similar to that characteristic of members of the genus *Alternaria*. The tip of the primary spore (Pl. 7, Dba) may bud to produce a sessile secondary spore (Pl. 7, Dbc) and this may produce a tertiary spore in the same way. Not infrequently, however, the tip of the spore (Pl. 7, Dbc) grows out into attenuated segments (Pl. 7, Dae) having approximately the diameter of the primary sporophore and obviously of a similar nature. These segments usually never attain any great length before proliferating a terminal spore (Pl. 7, Dbf); and as growth continues, fructifications result, consisting of superimposed spores and sporophoric segments, that may exceed 0.5 mm. in height. As the basal and distal segments of the spores not infrequently produce short sporophoric branches directed at oblique angles to the main axis (Pl. 7, Dab, ac, ad, af) and bearing one or more spores (Pl. 7, Dbd, be, bg), the fructifications usually are further complicated by the presence of a number of lateral sporiferous processes. It may be mentioned in this connection that the distinction between spore segment and sporophoric segment is not always well defined, but often may be partly obliterated, the fructification then being represented by a process of segments varying from 6 to 18  $\mu$  in thickness, and disarticulating at certain constricted septa marked by the presence of hila.

As far as the writer is aware, the parasite on *Cinna arundinacea* has not been described in literature. Atkinson (4), it is true, reported *Helminthosporium turcicum* as occurring on the leaves of this host in Alabama. While it is not impossible that Atkinson may have been dealing with the same fungus that causes injury to maize, there would seem to be much more probability in the assumption that this writer was confronted with the fungus under consideration, although with the exception of the pathological effects occasioned by them, the similarity between the two parasites can hardly be said to be a close one. Because of its tendency toward the formation of chains of spores in nature as well as in pure culture, the fungus on wood reed grass is named *H. catenarium*.



## DIAGNOSIS

***Helminthosporium catenarium*, n. sp.**

Attacking the foliage of *Cinna arundinacea*, L., where it causes the premature death of large areas of tissue, the tip and margin being usually most commonly affected.

Sporophores brown or olivaceous; emerging usually from the stomata, singly or in groups of 2; measuring 5 to 8 by 60 to 200  $\mu$ ; producing the first spore at a distance of 25 to 60  $\mu$  from base, and successive spores at intervals of 15 to 30  $\mu$ , the point of attachment marked by scars at the apices of pronounced geniculations.

Spores 1 to 10-septate, the septa sometimes associated with slight constrictions or irregularities in the contour of the thin peripheral wall; subhyaline to light yellowish, 14 to 18 by 30 to 200  $\mu$ , measured at their maximum diameter; the shorter ones usually straight, widest at the basal or second segment, tapering uniformly to approximately half the maximum diameter at tip; the longer ones often perceptibly crooked, irregular in diameter, frequently showing both a basal hilum and an apical scar, the apical scar marking the attachment of a secondary spore, and usually associated with a peculiar modification of the distal portion of the terminal segment, consisting in the prolongation of the latter at a uniform diameter representing the minimum width of the spore. Secondary spores or spores of a higher order of the same diameter, but usually considerably shorter, less frequently septate, or continuous. Both types germinating normally by production of 1 or 2 lateral germ tubes from basal segment, and a single lateral or oblique tube from terminal segment. Contour of basal end hemi-ellipsoidal, of distal end hemispherical; hilum and apical scar not protruding.

In pure culture on potato glucose agar, aerial mycelium white or dirty yellowish, present as dense erect tufts 2 to 5 mm. high at point of inoculation and as small flecks scattered sparsely over the surface; in either case consisting of sterile hyphae and an increasing number of conidial fructifications; the latter arising on the expanded terminations of hyphae not otherwise much modified, and consisting of a series of successively proliferated spores that may be either sessile or separated by intercalary, narrower sporophoric segments. The fructification frequently branching; as a result of the proliferation of lateral or oblique sporophoric processes from the basal or terminal segment of individual spores; and, less typically, sometimes consisting of miscellaneous processes of segments varying from 6 to 18  $\mu$  in thickness, and disarticulating at constricted septa marked by the presence of scars or hyla.

HABITAT.—Parasitic on *Cinna arundinacea* L. collected at Douglaston, N. Y., September, 1920.<sup>7</sup>

**HELMINTHOSPORIUM BROMI DIEDICKE—PYRENOPHORA BROMI (DIED.)**

*Helminthosporium gramineum* Rab. f. sp. *bromi* Diedicke olim 1902, in Centbl. Bakt. [etc.] Abt. 2, Bd. 9, p. 317-320.

*Pleospora bromi* Died 1903, in Centbl. Bakt. [etc.] Abt. 2, Bd. 11, p. 52-59.

*Pleospora trichostoma* (Fr.) Wint. f. sp. *bromi* Noack 1905, in Ztschr. Pflanzenkr. v. 15, p. 193-205.

The occurrence in Germany of a species of *Helminthosporium* parasitic on *Bromus asper* Murr. was recorded by Diedicke (28) in 1902. Krieger<sup>8</sup> later collected and distributed the same fungus on the leaves of *Bromus inermis* Leyss. It undoubtedly is on the latter host that the parasite is found most commonly in the United States, observations made by the writer in the vicinity of Madison, Wis., during the spring of 1920, pointing toward its general prevalence on this widely distributed host. Diedicke described the disease symptoms occasioned by the fungus so adequately that little can be added except in the way of corroboration.

*Helminthosporium bromi* is probably one of the earliest of all parasites affecting the grasses of our northern latitudes, to appear in spring, as

<sup>7</sup> Type specimens of all species described in this paper as new have been deposited in the following herbaria: Office of Pathological Collections, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.; Cryptogamic Herbarium, Harvard University, Cambridge, Mass.; herbarium, New York Botanical Garden, New York City; herbarium, Department of Botany, University of Wisconsin, Madison, Wis.

<sup>8</sup> KRIEGER, W. FUNGI SAXONICI. No. 1941 *Helminthosporium bromi* Died. [exsiccati]. 1903, 1905.

the first leaves that arise from the overwintering rootstock of *B. inermis* have barely completed their development before they begin to show the scattered spotting characteristic of its attack. (Pl. 8, Ab-c.) Each spot originates as a minute dark brown or black speck about which the chlorophyll gradually appears to break down, producing a yellowish or nearly colorless halo. Both the central spot and the surrounding zone usually increase considerably in extent, especially in a longitudinal direction, until the former may occupy an area 2 mm. in width and 6 mm. in length. On badly infected leaves the yellowed zones frequently run together as shown in Plate 8, B. Even more moderate infection, however, leads to a premature withering of the leaf (Pl. 8, Ad), beginning at the tip and proceeding downward, until the whole structure may be involved; for although the sheath is much less frequently spotted, it is not entirely immune, especially while the plant is still very young. After the leaf has become withered the sporophores make their appearance, first on or near the darkened areas, but later quite generally over the entire leaf. Emerging singly or in clusters of two (Pl. 8, Ea-c), most frequently between epidermal cells without much reference to the stomata, they measure 7 to 10  $\mu$  in diameter and usually 100 to 150  $\mu$  in length, although sometimes attaining a maximum length of 250  $\mu$ . From 2 to 6 septa usually are present. The geniculations associated with the production of successive spores are not generally very pronounced.

In the account given by Diedicke (28) the spores are described as 4 to 6 septate, 108 to 150  $\mu$  long, 13 to 20  $\mu$  in diameter, and exactly similar to those described and figured by Ravn (115), the supposed similarity presumably applying to *Helminthosporium teres*. Such a statement of the morphological features of the spores of the two species, it is quite impossible to substantiate by a comparison of American material, which reveals instead very decisive differences. The contrast in length between the two species is especially conspicuous, the spores of *H. bromi* measuring from 45 to 265  $\mu$  in length, examples exceeding 200  $\mu$  in length being of not uncommon occurrence. (Pl. 8 Ca-f.) Some difference in diameter also is perceptible, although this is much less pronounced, the spores of *H. bromi* varying commonly from 14 to 26  $\mu$ . As the number of septa in the spores of both species varies from 1 to 10, it is apparent that the individual segments in the spores of *H. bromi* exceed in length those of *H. teres* in approximately the same measure as do the spores. In *H. teres*, moreover, the spore often is conspicuously constricted at the septa, a modification absent or less strongly pronounced in those of *H. bromi* (Pl. 8, De). Related to these features are the specific peculiarities in the contour of the basal cell that have been mentioned in another connection, this contour being approximately hemi-ellipsoidal in *H. bromi* and hemispherical in *H. teres*.

In the conidia of *Helminthosporium bromi*, the hilum is represented by a scar, not especially conspicuous, and included entirely within the contour of the peripheral wall. As the peripheral wall consists of a relatively thin membrane, it is not surprising that the conidia show little resistance to unfavorable conditions. Even in actively sporulating material, microscopic examination reveals one of several dead segments in most of the spores, and when material is kept in the laboratory the proportion of dead segments or dead spores is very greatly augmented in the course of a week. When mounted in water, viable spores germinate

very promptly, producing lateral or oblique germ tubes indiscriminately from middle and end cells, the number of tubes originating from any particular segment not usually exceeding two. Anastomoses of newly proliferated germ tubes are not infrequent, the germination of spores lying in juxtaposition, thus often yielding scalariform figures united by several hyphal connections. (Pl. 8, Db-c.) As has been pointed out previously, when the fungus is cultivated on potato agar media, anastomosis of hyphae is associated often with the production, below the surface of the substratum, of numerous groups of inflated segments. Of the latter, a small proportion develop into subspherical sclerotia, usually from 0.2 to 0.5 mm. in diameter, the presence of which in agar cultures (Pl. 9, C) is characteristic of the fungus, and which, judging from their size and structure, doubtless represent immature perithecia.

In Germany, Diedicke (28) noted the appearance on *Bromus asper* of the sclerotia or young perithecia as early as July 26. This date corresponds quite closely with their appearance in southern Wisconsin. When fully matured, they consist of a subglobose portion imbedded within the somewhat distended leaf tissue, usually from 0.3 to 0.4 mm. in diameter and tapering into an irregular ostiolar beak protruding approximately 0.1 mm. above the broken epidermis. A variable number of septate, tapering, sterile bristles usually are to be found near the tip of the ostiolar modification; and although it is not improbable that in some seasons conidiophores may be produced abundantly from the perithecium as in *Pyrenophora tritici-repentis* and *P. teres*, only a sparse production was observed during the season of 1920.

The development of the asci appears to be delayed until spring, beginning evidently with the warmer weather usually experienced in our northern latitudes late in February or early in March, and proceeds slowly, until toward the middle of April they readily discharge their spores when mounted in water. In all morphological details the asci closely resemble those of *Pyrenophora tritici-repentis*, although they usually seem to be somewhat larger. The difference, which is not pronounced, becomes, perhaps, most evident during the process incident to spore discharge, as the asci of the parasite on brome grass appear to undergo perceptibly greater distention before the outer membrane is burst than the asci of the congeneric parasite on quack grass. Plate 9, D, representing an ascus partly distended, measuring 300  $\mu$  in length and 65  $\mu$  in diameter, shows the 8 spores in distichous arrangement immersed in granular epiplasm, the thickened ringlike modification at the apex, and the short well-defined stipe common to both species.

The ascospores, light brown in color and measuring 20 to 30  $\mu$  by 45 to 72  $\mu$ , are uniformly divided by 3 transverse septa. Longitudinal septa may be absent, or one or both of the middle segments may be further divided by a longitudinal wall. Germination takes place by the production of a germ tube from several or all of the segments. Cultures of the fungus derived from ascospores differ in no particular from those derived from conidia.

Because of the distinctive and extraordinarily large conidia characteristic of the parasite and its production of sclerotia on various kinds of agar media, the writer agrees with Diedicke in regarding the fungus as an independent species. In this connection it may be mentioned that Diedicke (28) first reported it as a biological species of the stripe fungus, a disposition which Noack (95), who recognized *Pleospora trichostoma* as the ascigerous stage of *Helminthosporium gramineum*, later sought to

maintain. The ascigerous stage, it must be admitted, is morphologically not readily distinguished from related congeneric forms. The fungus, in any case, would seem to be referable to *Pyrenophora* rather than to *Pleospora*, if, indeed, the former is to be maintained as a separate genus.

#### HELMINTHOSPORIUM GIGANTEUM H. & W.

Heald and Wolf, in 1911, described (54) and later also (55) figured a species of *Helminthosporium* on Bermuda grass (*Cynodon dactylon* L. = *Capriola dactylon* [L.] Kuntze) collected at Falfurrias, Tex., where it was found associated with a disease—

characterized by the presence of numerous yellowish or pale straw-colored spots, 0.5 to 1 mm. wide, by 1 to 4 mm. long, longitudinally elongated, and with a narrow brown border. The spots are generally absent from the leaf sheath, and when numerous they may become confluent on the lamina and thus cause somewhat extended dead areas.

The fungus, which was named quite appropriately *Helminthosporium giganteum*, was further characterized as follows:

The conidiophores are dark brown, many-septate, 9 to 12 by 200 to 400  $\mu$ , with a slightly bulbous base; the spores are elongated, cylindrical with slightly tapering ends, 5-septate, pale brown, densely granular contents, 15 to 21 by 300 to 315  $\mu$ .

The writer collected the same parasite on Bermuda grass at various times during the months of February, March, and April, 1921, near Fort Myers and Wauchula, Fla. During February and March, especially in the vicinity of Fort Myers, the fungus occurred in considerable abundance, old spots bearing sporophores being found scattered generally over the foliage of the host. Fresh lesions providing material suitable for study were almost entirely absent at that time, a circumstance attributable apparently to the condition of the host; for the latter, although green, was not actively growing and the foliage, moreover, was everywhere severely affected by *Puccinia cynodontis* Desm. and *Helminthosporium cynodonti* Marignoni. About the middle of April, several weeks after the resumption of active vegetative growth, the more recently unfolded leaves of *Cynodon dactylon* began to show newly developed lesions associated with conidiophores and conidia in a living state. At nearly the same time altogether similar foliar spots made their appearance on goose grass (*Eleusine indica* [L.] Gaertn.). Microscopical examination revealed no morphological difference in the fructifications present on the two graminaceous species.

On July 13, 1922, a stand of Bermuda grass near Bladensburg, Md., was observed to be attacked by the fungus with unusual severity. Many of the leaves had been killed altogether, and of those that remained functional nearly all bore scores of discrete or confluent spots, or extensive whitened areas which often involved altogether from one-fourth to one-third of the tissues of the individual foliar organs. Plants of *Agropyron repens* distributed in the Bermuda grass likewise bore a sprinkling of the foliar spots characteristic of the fungus, although in smaller quantity. On microscopical examination the similarity of the abundant fructifications on both Bermuda and quack grass collected in Maryland to those of the Florida parasite was readily apparent. *Agropyron repens* and *Eleusine indica*, therefore, represent additional hosts of the interesting fungus described by Heald and Wolf (55).

The foliar lesions bear a good deal of resemblance to those produced, for example, by *Helminthosporium leersii*. They first become visible as minute brown spots in green and otherwise healthy tissue. (Pl. 10, A.)

Soon these spots increase in length and width, the center in the meantime fading to almost white or light straw color. In this way the appearance described by Heald and Wolf (54) is brought about. (Pl. 10, A, B.) At this stage the lesions are of the simple "eye-spot" type. Under certain conditions, apparently when the parasite is thriving most luxuriantly, the diseased areas become greatly enlarged, sometimes occupying the entire width of the leaf blade of Bermuda grass for a distance of one centimeter or more. When such development occurs the dead areas usually show a number of roughly concentric brown markings, evidently corresponding to successive positions of the margin, and giving the areas a distinctively zonate aspect. With the multiplication of the smaller lesions and the extension of the larger ones, a large proportion of the leaves are killed outright long before the end of the season. Previous to this the sporophores of the fungus make their appearance singly or in pairs over the larger dead regions always at some distance from the margin of the surrounding green tissue. The conidiophores are distributed rather sparsely in comparison with the crowded spacing of the homologous structures of many congeneric forms, although not actually few in number.

The sporophores of *Helminthosporium giganteum* (Pl. 10, Ea, b) are the largest of those of any species studied by the writer, the measurements of the Florida material agreeing well with those given in the original specific description. The septa are mostly spaced with considerable regularity, at intervals varying commonly from 25 to 40  $\mu$ . Usually the scar marking the point of attachment of the first spore is found 140 to 250  $\mu$  from the base, and successive scars associated with geniculations, usually not pronounced, occur at intervals of approximately 40  $\mu$ .

The conidia (Pl. 10 Ca-d), which are produced in relatively small numbers, are easily the most massive of those of any species of *Helminthosporium* hitherto described, and are probably among the very largest produced by any group of fungi. Individual spores were found exceeding considerably in length even the generous measurements given by Heald and Wolf, the one figured in Plate 10, Cc, for example, measuring 385  $\mu$  in length and 21  $\mu$  in diameter. The volume of a spore of such dimensions is several hundred times greater than the volume of spores of molds that are not by any means regarded as minute fungi, while on comparison with some of the smallest types, like species of *Actinomyces*, ratios approximately 1 to 300,000 may be obtained. The writer has found it possible, after staining with eosin, to make out with the naked eye, spores approaching the dimensions given.

Besides being the largest, the conidia of *Helminthosporium giganteum* also, perhaps, are the shortest-lived conidia of any species of *Helminthosporium* discussed in this paper. If the spores from a group of fructifications which are still actively proliferating new conidia are mounted in water, a large proportion will be found to contain one or more dead segments. After spore production in a group of fructifications ceases all of the conidia will contain dead segments, and in a relatively brief period, probably not exceeding two weeks, only a small number will still show living segments. To this lack of longevity, together with the production of spores in small numbers, the comparatively restricted occurrence of the parasite probably may be attributed.

The spores of *Helminthosporium giganteum*, while alive, are altogether hyaline, colorless, and filled with homogeneous protoplasm. The characterization of these structures by Heald and Wolf in the words, "pale

brown, densely granular contents," would seem to indicate that these authors very probably drew their description from dead material. The peripheral wall is thin as in other species with subhyaline spores, and sometimes shows a perceptible constriction at the septa, the latter varying in number usually from 2 to 6 and delimiting segments ranging up to  $80\ \mu$  in length, and from  $16$  to  $25\ \mu$  in diameter. The ends of the spores are rounded off abruptly, showing usually a hemispherical or hemi-ellipsoidal contour. The basal end is modified by the presence of a minute dark apicular projection at the apex of a small, faintly delimited, obtusely conical part, which, while the spore is still attached, fits into a depression in the center of the scar on the sporophore.

When the spores are placed in water they germinate very promptly, the germ tubes being proliferated sometimes singly and indiscriminately from end or middle segments (Pl. 10, Da, c); or more typically in groups of 3 or 4, usually from both basal and apical ends (Pl. 10, Db), or less commonly from one or more intermediate segments (Pl. 10, Dc). In any case the germ tubes are of unusual width, measuring 6 to  $10\ \mu$  in diameter, and grow with remarkable rapidity. Germination is associated with a change in the protoplasm from an apparently homogeneous to an abundantly vacuolated structure. It may not be superfluous to add that the spores filled with "densely granular contents" never germinate, and segments exhibiting such structure, always together with a swollen peripheral wall, similarly remain inert.<sup>9</sup>

#### HELMINTHOSPORIUM DICTYOIDES, N. SP.

During the latter part of June, 1920, the writer observed a disease on meadow fescue (*Festuca elatior*, L.) which seemed to be widely prevalent in the region about Washington, D. C., scarcely any stand of this grass being entirely free from it. As meadow fescue, although not one of our most highly prized forage plants, is nevertheless of not inconsiderable economic importance, and is besides very generally distributed, an effort was made to determine whether the disease occurs also in other sections. In the vicinity of New York City, from early in July to the end of August, it was found to be so common on *F. elatior* that the very characteristic and conspicuous lesions could, in the absence of an inflorescence, be quite safely used to distinguish this species of grass from grasses having somewhat similar foliage as, for example, *Bromus inermis* or *B. secalinus* L. Especially severely affected material was found near Port Washington on Long Island, where the dampness of the atmosphere incident to proximity to the sea, may have favored the progress of the malady. Other collections of diseased material were made at Stamford, Conn., August 2, 1920; Norwood, Mass., November 7, 1920; Lisbon Falls, Me., July 24, 1921; Annapolis, Md., October 15, 1921; and at numerous stations in Maryland, Virginia, and the District of Columbia near Washington, D. C., from early in August until late in October, 1921. Indeed, the writer has never failed to find the disease present to a greater or less extent on meadow fescue in all the localities in the New England and Middle Atlantic States that he has had occasion to visit.

The symptoms of the trouble, a brief account of which was published in 1922 (32), are very similar to those induced by *Helminthosporium*

<sup>9</sup> Since this account was written the writer has had opportunity to study the mode of development of the fungus in greater detail and on a considerable number of additional hosts. The results have been partly indicated in a brief note (33), and will be published in more complete form in a later paper.

teres on barley. The newly affected green tissues show abundant brownish discoloration in irregular patterns, within which may be recognized a network of darker longitudinal and transverse linear streaks. (Pl. 11, A, C.) The minute reticulate design formed by the latter is, in well developed cases, more extensive and pronounced than in any specimens of barley affected with netblotch which the writer has ever seen. After a considerable portion of the leaf blade has been involved, it gradually withers and dies, the withering beginning at the tip and proceeding toward the base. (Pl. 11, A.) In 1920, in the neighborhood of New York City, such destruction of foliage continued throughout nearly the entire season, and caused an amount of damage that appeared to be far from trivial. Indeed, the writer is inclined to believe that the malady, which may conveniently be designated like that caused by *H. teres*, as netblotch, is the most serious parasitic trouble affecting meadow fescue in our northern latitudes.

On examining the withered portions of affected leaf blades, the cause of the disease is readily recognized as a species of *Helminthosporium*. To *H. teres*, however, the fungus shows no close similarity, the spores of the barley parasite having dimensions so much greater as to preclude any possibility of confusing the two forms. It shows a much greater degree of similarity to *H. gramineum*. The sporophores, as in the stripe fungus, are found in groups larger than in most congeneric species, the number in a group varying usually from 2 to 6 (Pl. 11, Ea-i). On the other hand, the basal enlargement characteristic of the sporophores of *H. gramineum* appears to be less pronounced in the homologous structures of the form on meadow fescue. The spores of the two fungi possess some characteristics in common, but exhibit quite distinctive specific differences as well. Thus they agree in color, in both species varying from subhyaline and colorless when newly proliferated, to distinctly yellowish when fully mature; and on measuring show an approximately equal range in length. However, the spores of *H. gramineum* are appreciably greater in diameter, and, while manifesting a tendency to taper toward the apex, do not depart very greatly from a straight-cylindrical type, whereas those of the parasite on *Festuca elatior* (Pl. 11, Ba-g) more frequently show a very pronounced diminution in diameter from the base to the tip, and in only relatively few instances approach an approximately cylindrical shape. In germinating the conidia of the fungus on meadow fescue do not generally produce germ tubes indiscriminately from both the end and the middle segments, but typically give rise to a lateral or oblique germ tube from one or both end segments (Pl. 11, Dc-k), although, less frequently, one or more germ tubes may be produced from a middle segment (Pl. 11, Da-b). It also may be mentioned that the spontaneous development from primary spores of short sporophores bearing secondary spores, characteristic of the barley stripe fungus, has not been observed in any material of the parasite on meadow fescue.

Another congeneric form with which the species of *Helminthosporium* on *Festuca elatior* might possibly be confused is the one described in this paper as *H. siccans* that occurs on *Lolium multiflorum* Lam. and *L. perenne* L. The latter on comparative examination, however, is readily distinguished by the appreciably larger dimensions, conspicuously darker coloration, and less tapering form characteristic of its conidia. These conidia when altogether mature possess, moreover, a considerably thicker peripheral wall, and, in germinating, typically produce two germ tubes from each of the end segments instead of one.

The literature contains, as far as the writer is aware, only two references to the occurrence of a species of *Helminthosporium* on *Festuca elatior*. The earliest is that of Diedicke (29), who recorded his discovery of a parasitic form that produced a local infection similar to the type of infection brought about by *H. bromi*, *H. teres*, and *H. avenae*. As this writer gave no further details concerning either the fungus or the disease, it is not possible to determine whether or not he was dealing with the parasite found widely distributed in the Middle Atlantic States. In a later reference by Pammel, King, and Bakke (104), is reported from Iowa the occurrence on *F. pratensis* (= *F. elatior* L.) of a species of *Helminthosporium* having spores similar to those of *H. sativum* and producing on its host a leaf spot closely resembling late blight of barley, although less severe. Material deposited by these authors in the Office of Pathological Collections, Bureau of Plant Industry, was examined by the writer. This examination revealed no reason why the fungus should not be referred to *H. sativum*. At any rate the Iowa fungus represents an entirely different organism from the one under consideration, which would appear to merit recognition as a new species. On account of the reticulate pattern characteristic of the foliar lesions the specific name *dictyoides* is suggested.

#### DIAGNOSIS

#### *Helminthosporium dictyoides*, n. sp.

Occurring on *Festuca elatior* L., on which it causes a moderately destructive disease of the foliage, with symptoms very similar to those of barley attacked by *Helminthosporium teres*; newly infected leaves showing irregular brownish areas marked with dark longitudinal and transverse streaks forming a delicate reticulate pattern. Affected leaves later withering, the withering beginning at the tip and progressing to the base of the blade.

Sporophores dark brown or olivaceous; emerging singly or in groups of 2 to 6 from stomata or between epidermal cells; measuring usually 6 to 8  $\mu$  in diameter and 70 to 150  $\mu$  in length; 3 to 6-septate, the septa generally occurring at intervals of 10 to 30  $\mu$ ; producing first spore usually at a distance of 50 to 100  $\mu$  from base; points of attachment of successive spores marked by moderately or strongly pronounced geniculations.

Conidia subhyaline and nearly colorless when newly proliferated, to yellow when fully matured; typically straight; maximum diameter usually at basal segment, 14 to 17  $\mu$ ; tapering uniformly and very perceptibly to apical segment, the latter in long spores frequently not exceeding 8 to 9  $\mu$  in diameter, in short ones usually of greater diameter; more rarely approximately cylindrical, or short ellipsoidal. Length 23 to 115  $\mu$ , usually 50 to 70  $\mu$ , 1 to 7 septate, typically 3 to 5 septate, the septa not associated with perceptible constrictions except occasionally, and then constrictions most frequently present only at the basal septum; length of segments 7 to 24  $\mu$ , typically 12 to 15  $\mu$ . Contour of basal end hemispherical, of apical end hemispherical or hemi-ellipsoidal; peripheral wall or exospore uniformly thin, and entirely including the hilum within its contour. Germinating typically by two germ tubes, one from each end segment and produced usually at right or oblique angle to axis of spore; rarely by one or more germ tubes from intermediate segments.

**HABITAT.**—Collected at Brooklyn and Port Washington, N. Y.; Stamford, Conn.; Norwood, Mass.; Lisbon Falls, Me.; Washington, D. C.; Kensington and Annapolis, Md.; and Falls Church, Va. Apparently found wherever the host occurs in the Middle Atlantic and New England States.

#### HELMINTHOSPORIUM SICCANS, N. SP.

In the latter part of June, 1922, the writer observed that the Italian rye grass, *Lolium multiflorum* Lam, in the experimental farm at Arlington, Va., was very generally affected by a leaf spot disease. The trouble was found to occur on the foliage of both young and more nearly mature plants, being manifested by the appearance of minute, longitudinally



elongated, dark brown spots, measuring usually 0.1 to 0.3 mm. in width and 0.2 to 1.0 mm. in length, although sometimes apparently as a result of coalescence, attaining dimensions several times larger. Many of the more severely affected leaf blades bore more than a hundred of these localized discolorations and frequently more than a dozen could be distinguished on the sheath, mostly near its juncture with the blade. (Pl. 12, Ab.) Owing to the large number of spots often present on a single foliar organ, an appearance somewhat suggestive of netblotch is brought about, but as distinct transverse markings have not been observed, the reticulate pattern characteristic of leaves of barley and meadow fescue attacked by *Helminthosporium teres* and *H. dictyoides*, respectively, is not evident. It may be mentioned that the most distinctive sharply defined discolorations have usually been found on leaves of younger plants, while on the leaves of plants attacked after the heading stage the discoloration frequently appeared to be somewhat suppressed. In any case, however, the organs attacked soon turned yellow at the tip and withered, the withering eventually involving the sheaths as well as the blades.

Microscopical examination of leaves that had succumbed to the malady revealed an abundance of fructifications typical of the genus *Helminthosporium* emerging from the dead tissues. Although the symptoms of the disease caused by it are quite different from those of the stripe disease of barley, the fungus shows a strong resemblance to *H. gramineum*. The spores of the two species are nearly similar in shape, being usually straight and cylindrical or tapering toward the tip. The tendency toward tapering, to be sure, is more pronounced in the conidia of the parasite on Italian rye grass, which, moreover, when fully mature, are of a brown or brownish olivaceous color, appreciably darker than the yellow fuliginous hue characteristic of the spores of the stripe fungus. With this deeper coloration is associated a peripheral wall, decidedly thicker than the spore wall of subhyaline-spored types but inferior to that of species possessing olivaceous spores of the type of *H. sativum*. The conidia of the form on *Lolium multiflorum* are perceptibly longer than those of *H. gramineum*, and a proportional inequality obtains in regard to the spore segments of the two species.

Germination in water normally takes place in a manner very similar to the germination in *H. gramineum*; that is, by the production typically of one or two germ tubes from both apical and basal segments and a single tube from several or all intermediate segments, although occasionally two tubes may be proliferated from an intermediate segment. The production of sporophoric processes by conidia under natural conditions, while not uncommon, is not as frequent in the fungus on *L. multiflorum* as in the stripe fungus, and apparently usually comes to an end with the production of a single secondary conidium.

The form on Italian rye grass shows considerable resemblance to *Helminthosporium dictyoides* on meadow fescue, not only in general pathological habit but in morphological details as well. The conidia are largely of the same straight cylindrical or tapering form. They are noticeably larger, however, more frequently septate, and when fully mature darker and provided with a thicker peripheral wall. The mode of germination also shows some difference, the parasite on *Lolium multiflorum* being usually more profuse in the production of germ tubes. With the form described in this paper as *H. stenacrum* occurring on *Agrostis stolonifera* the fungus is not readily confused, being distinguished

by its smaller dimensions, thicker peripheral wall, and absence of attenuated apical prolongation.

Although not much importance can be attributed to the length of the conidiophores as a diagnostic characteristic, it may not be superfluous to mention that in the species under consideration these structures show a rather usually wide range in this dimension. This variability apparently is less due to differences in length of intervals between the points of insertion of successive spores than to pronounced differences in length of the sterile portion below the insertion of the first conidium. It is not difficult to suppose that changes in environmental conditions are responsible for either greater or lesser development of the sporophore preliminary to the proliferation of the first conidium, resulting in conditions like those illustrated in Plate 12, Fe-j, on the one hand, and like those figured in Plate 12, Fa-d, on the other.

A fungus quite indistinguishable from the form on Italian rye grass was found to occur abundantly in Virginia and Maryland on the closely related host, *Lolium perenne*. An examination of numerous specimens of diseased perennial rye grass collected near Annapolis in May, 1921, and in the vicinity of Washington, D. C., during the months of May and June, 1922, revealed no constant or significant morphological characteristics by which the form parasitic on this forage crop could be distinguished from the parasite on Italian rye grass. In the absence of any cross-inoculation work the writer is inclined to regard the fructifications on the two hosts as belonging to the same species of *Helminthosporium*. It must be noted, however, that the attack of the fungus on *L. perenne* is usually not associated with the conspicuous spotting of the leaves readily observed on affected foliage of *L. multiflorum*, the discoloration being generally less evident and in many instances scarcely demonstrable. In the latter event, the withering of the foliage due to the parasite is, without microscopical examination, not very easily distinguished from the vegetative decline associated with drought or normal ripening.

As a large proportion of the leaves of the two species of *Lolium* are killed prematurely, it is safe to assume that the parasite interferes with the development of the plants sufficiently to cause appreciable economic loss. According to the writer's observations, the leaf disease attributable to the parasite constitutes the most destructive fungus trouble affecting the two valuable forage grasses in Maryland, Virginia, and the District of Columbia. That it has hitherto apparently escaped the attention of American pathologists is indicative more, perhaps, of neglect of diseases destructive to the graminaceous forage crops than to a possibly limited distribution.

It is interesting to note that in 1903 Diedicke (29) made mention of the occurrence of a species of *Helminthosporium* on *Lolium perenne*, causing a local infection similar to that occasioned, for example, by *Helminthosporium bromi* or *H. teres* on their respective hosts. No further description was given, and this investigator, after failing to connect the fungus with any ascigerous stage, apparently paid no further attention to it. Whether the American fungus is the same as that observed by Diedicke in Germany is a question open to conjecture. In any case, it appears not to have been described; and it evidently is distinct from the other species parasitic on grasses studied by the writer. It appears expedient to recognize it as a species, for which, because of its pathological effect on the foliage of its hosts, the specific name *siccans* is proposed.

## DIAGNOSIS

**Helminthosporium siccans**, n. sp.

Occurring on *Lolium multiflorum* Lam. (type) and *L. perenne* L., on which hosts it causes a moderately destructive disease of the foliage, producing typically numerous dark brown longitudinally elongated spots, usually measuring 0.1 to 0.3 mm. in width by 0.2 to 1.0 mm. in length that by coalescing often appear as discolored areas several times larger. Affected leaves later dying, the withering beginning at the tip of the blade and eventually involving the sheath wholly or in part.

Conidiophores olivaceous, emerging usually singly, less frequently in pairs, and rarely in groups of three from stomata or more especially from between epidermal cells on the vascular ridges; measuring 7 to 9  $\mu$  in diameter by 50 to 300  $\mu$  in length; 1 to 9 septate, the septa inserted at intervals of 15 to 90  $\mu$ ; producing first conidium at a distance of 50 to 250  $\mu$  from base; points of attachment of successive conidia at angles of geniculate irregularities occurring at intervals of 5 to 30  $\mu$ .

Conidia subhyaline or light fuliginous when newly proliferated, later becoming yellow, brownish, or brownish olivaceous, never dark olivaceous like *Helminthosporium sativum*; when fully mature provided with a moderately thick peripheral wall; typically straight or slightly curved; measuring usually 14 to 20  $\mu$  in diameter by 35 to 130  $\mu$  in length; usually subcylindrical, or tapering slightly or more markedly toward apex, the distal segment often not exceeding 10  $\mu$  in diameter, or rarely greater in diameter at the distal end than at the base. Apical and basal ends abruptly rounded off, the contours at the ends being approximately hemispherical. Hilum moderately conspicuous included within contours of peripheral wall. Germinating by the production of germ tubes from intermediate as well as end segments, the basal and distal segments both usually participating in the process by the proliferation of 1 or 2 lateral or oblique germ tubes. Of the intermediate segments one, several, or all may produce one or rarely two lateral germ tubes. Under natural conditions germination by the production of one or less frequently two sporophoric processes, each bearing usually a single conidium, is not uncommon.

HABITAT.—Collected in May and June near Annapolis, Md.; Baltimore, Md.; Rockville, Md.; Kensington, Md.; Washington, D. C.; Arlington, Va.; Mount Vernon, Va.

**HELMINTHOSPORIUM STENACRUM**, N. SP.

On specimens of *Agrostis stolonifera* L. collected at various times in September, October, and early November, 1920, in southwestern Connecticut, especially in the vicinity of Stamford and Norwalk, the writer found a well characterized species of *Helminthosporium* occurring with some regularity. The fructifications were found on dry withered leaves that formed a considerable proportion of the foliage. Owing to the relatively small size of the foliar organs concerned, and the absence of any pronounced discoloration, it was not possible to determine definitely whether the dying of the leaves was due to the presence of the fungus or to other causes. In general, the distribution of the conidiophores on the dead tissues suggested a course of events somewhat similar to that exemplified by the parasitism of *H. turcicum* in ordinary seasons—development of the fungus on leaves already reduced in vitality as a result of normal maturation, followed by the production of fructifications some time after their death.

The sporophores distributed rather sparsely over the surface of the withered leaves are moderately large structures of the same general type as those of *Helminthosporium teres*, although usually more abundantly septate and less frequently occurring in groups. (Pl. 13, Ca-d.) In width, color, character of peripheral membrane, general shape, and mode of germination, the spores show some similarity to those of *H. gramineum*, *H. teres*, and *H. avenae*. (Pl. 13, Aa-r, Ba-c.) In length, however, they are decidedly superior to the spores of *H. gramineum*, and in an approximately equal degree inferior to those of the other two species. While in newly proliferated spores the peripheral wall may exhibit slight constrictions at the septa, the contour of fully mature spores usually is

altogether smooth, a detail in which the species shows more similarity to *H. siccans* and *H. gramineum* than to the barley net-blotch fungus. No tendency toward the production of secondary spores, such as is manifested in *H. gramineum* and *H. catenarium*, has ever been observed. As in *H. catenarium*, however, the distal portion of the spore is frequently produced into a somewhat constricted apical prolongation. Apparently such modification is the result of development taking place subsequent to the proliferation of the spore in its original condition, and thus, in a sense, is of secondary origin. In any case, the apical extension is characterized by a conspicuous paucity of septa, such cross walls as are found present usually appearing to have developed tardily, as the delimited segments frequently have not contracted away from one another along the edge of the plane of contact.

In pure culture on artificial substrata the fungus produces abundant, although not especially characteristic, growth. Normal sporulation on the media ordinarily employed in laboratories has not been observed, although on tap water agar a relatively sparse production of somewhat small, atypical fructifications was brought about. The imbedded mycelium shows abundant anastomosis resulting in numerous complexes of inflated cells similar to those produced, for example, by *Helminthosporium teres*, *H. bromi*, and *H. tritici-repentis*; and, if the analogy is not misleading, pointing toward the existence of an ascigerous stage.

The fungus appears to be quite distinct from any graminicolous species of *Helminthosporium* hitherto described; as well as from several undescribed forms which the writer has collected on both wild and cultivated members of the genus *Agrostis*. Because of the somewhat attenuated distal prolongation characteristic of many of the spores, the specific name *stenacrum* is suggested.

#### DIAGNOSIS

#### *Helminthosporium stenacrum*, n. sp.

Occurring on withered leaves of *Agrostis stolonifera* L.

Sporophores dark olivaceous, emerging singly or in pairs, usually between adjacent epidermal cells; measuring 7 to 10  $\mu$  in diameter, and 80 to 250  $\mu$  in length; 3- to 10-septate, the septa occurring at intervals of 10 to 35  $\mu$ ; producing the first spore usually 80 to 150  $\mu$  from the base, the points of attachment of successive spores occurring at well-defined geniculations.

Spores subhyaline to yellowish when fully mature; 15 to 23 by 53 to 135  $\mu$ ; sub-cylindrical with hemispherical or hemi-ellipsoidal ends, or widest somewhat below the middle and tapering moderately toward the ends; the apical portion sometimes produced into a somewhat narrowed distal prolongation; 1 to 11 septate, the septa not associated with constrictions, or marked by barely perceptible constrictions. The peripheral wall thin and including the dark hilum within its contour. Germinating by the production of germ tubes from several or from all segments, the intermediate segments proliferating usually not more than one tube, the end segments occasionally giving rise to two or even three tubes.

HABITAT.—Collected near Stamford, Conn., and Norwalk, Conn., in September, October, and November, 1920.)

#### HELMINTHOSPORIUM DEMATIOIDEUM BUBÁK & WRÓBLEWSKI

In 1916, Bubák and Wróblewski (18) described as *Helminthosporium dematioideum* a fungus occurring in Galicia on the glumes and paleas of sweet vernal grass, *Anthoxanthum odoratum* L.

Caespitulis minutis, dispersis, pulverulentis, atris. Mycelio dematioideo, repente, atrobrunneo. Conidiophoris cylindricis, 25-60  $\mu$  longis, 5-6  $\mu$  latis, subtorulosis, septatis, brunneis.

Conidiis cylindraceo-oblongis, 38-42  $\mu$  longis, 9-13  $\mu$  latis, maturis 3 septatis, utrinque late rotundatis, flavobrunneis, crasse tunicatis, levibus.

The description applies moderately well to a fungus found occurring in abundance on dead leaves of sweet vernal grass collected near Washington, D. C., June 20, 1920, and at various times throughout July, 1920, near Port Washington, N. Y. On the inflorescence, the fungus, it is true, also was present, but in much smaller amounts than on the foliage. It was also found on withered foliage of *Agrostis alba* L. (= *A. palustris* Huds.) and *Agrostis perennans* (Walt.) Tuckerm., collected during July and August, 1920, near Brooklyn, N. Y., and Norwalk, Conn., not infrequently on the same leaves that bore also fructifications of one or another of the two apparently undescribed larger-spored species of *Helminthosporium* occurring on these grasses. As the presence of the fungus was in no case associated with local discoloration of the foliar tissue that served as substratum, it was not possible to determine definitely its relationship as a saprophyte or possible parasite. It may be said, however, that the organism, although fairly common on the different species of *Agrostis* mentioned, was not present in sufficient quantity to justify the belief that it was the main cause of the premature withering, abundantly observed during the season of 1920. On the other hand, the manner of its occurrence on *Anthoxanthum odoratum* is not such as to exclude the possibility of a parasitic relation. For in some collections every leaf appears to bear fructifications of the fungus, sometimes, indeed, in great abundance and not infrequently to the approximate exclusion of other fungi. Owing to the small size of the leaves of sweet vernal grass, and the consequent difficulty in recognizing possibly pathological withering from the withering normally taking place during the later stages in the life of the plant, the presence or absence of a parasitic relation could be established perhaps only by well controlled infection experiments.

The principal circumstance that might suggest a possible lack of identity of the European and American forms, is the recorded occurrence of the former only on the inflorescence of *Anthoxanthum odoratum*, while the latter, although not absent from the inflorescence, is certainly much more abundant on the foliage. As sweet vernal grass is an early maturing species and as the material from which the description of the Galician fungus was drawn appears to have been collected in October, it is not improbable that the leaves of the plant were rather poorly represented in the type specimens if, indeed, not altogether missing. Thus the American fungus may at least provisionally be referred to *Helminthosporium dematioideum*, although a few remarks concerning its morphology may not be out of place, especially in view of the brevity of the diagnosis given by Bubák and Wróblewski.

The sporophores (Pl. 14, Da-c), as indicated in the original description by these authors, measure only 5 to 6  $\mu$  in width and, compared to most graminicolous congeneric species, are generally decidedly short, although not infrequently exceeding 60  $\mu$  in length (Pl. 14, Db, c), and even in some instances measuring three times as much (Pl. 14, Da). They usually are found singly or in pairs, rarely in larger groups; are septate usually at intervals of 10 to 25  $\mu$ ; and often can be collected bearing 6 to 8 spores *in situ*. (Pl. 14, Db.) The spores (Pl. 14, Ba-h) are distinctly yellowish when fully mature, from 2 to 6 times septate, and measure 8.5 to 14 by 18 to 48  $\mu$ , although the range in septation and in dimensions given by Bubák and Wróblewski may be regarded as typical. In shape they are generally subcylindrical or tapering perceptibly toward the base. The proximal septum is frequently, but not constantly, associated with a

constriction; the basal, like the distal end, usually presents a hemispherical contour within which the small dark hilum marking the point of attachment may be readily recognized. Germination normally proceeds by the production of one to three germ tubes from the basal cell, at positions approximately midway between hilum and basal segment, not usually immediately adjacent to the hilum. (Pl. 14, Ca, Cc-g.) Other modes of germination, as, for example, the production of a germ tube from the apical segment (Pl. 14, Cb), may be regarded as abnormal and usually are attributable to the death of the basal segment as evidenced by the concave contour of the cross wall in contact with the adjacent living segment.

#### HELMINTHOSPORIUM TRISEPTATUM, N. SP.

In collections of velvet grass (*Notholcus lanatus* [L.] Nash [= *Holcus lanatus* L.]) made near Port Washington, Mineola, Douglaston, and other localities on the western end of Long Island during the months of July and August, 1920, a fungus related to the plants usually referred to the genus *Helminthosporium* was found occurring quite commonly on withered or withering leaves. (Pl. 14, E.) As the form is of a type somewhat different from the majority of the species of *Helminthosporium* on grasses, and does not appear to have been described hitherto, a brief account of it may not be out of place.

The sporophores (Pl. 14, Ha-c) are scattered relatively sparsely between the long hairs that constitute the abundant pubescence characteristic of velvet grass. They are distinguished not only by being relatively long, but also by the presence of ring-like thickenings of the peripheral wall immediately below the points of attachment of the successive spores. The upper portion of the sporophore may thus present a more or less distinctly moniliform contour, within which the lumen maintains an approximately uniform diameter.

Compared to the other species of *Helminthosporium* discussed in this paper, the spores (Pl. 14, Fa-f) of the fungus on velvet grass show perhaps the greatest degree of constancy with regard to morphological features. Associated with the dark olivaceous color is a peripheral wall not exceeded in thickness by that of any of the related forms described in this paper. At the basal end, however, it decreases uniformly in thickness toward the hilum, where the curved inner and outer contours appear to become tangent to each other. This condition is present also in other forms as, for example, in *Helminthosporium monoceras*, where, however, the wall is similarly attenuated at the apical end, a difference reflected in mode of germination characteristic of the species. For, whereas *H. monoceras* germinates from both ends, in the form under consideration only the basal segment normally participates directly in the process. (Pl. 14, Ga-e.)

The fungus appears to be mainly, if not wholly saprophytic. It is referred to the genus *Helminthosporium* rather than to *Brachysporium*, as in well developed spores (Pl. 14, Fe) the length is somewhat greater in proportion to the diameter than generally appears to be true in fungi assigned to the latter genus. The specific name *triseptatum* is suggested because of the number of septa characteristic of typical spores.

## DIAGNOSIS

***Helminthosporium triseptatum*, n. sp.**

Fructifications scattered sparsely on withering leaves of *Notholcus lanatus* (L.) Nash (= *Holcus lanatus* L.); usually not visible macroscopically or associated with visible alterations in the substratum.

Sporophores arising singly or in pairs, dark olivaceous, 6 to 8 by 200 to 400  $\mu$ ; usually 6 to 11 septate, the septa occurring at intervals 18 to 40  $\mu$  in length, averaging approximately 25  $\mu$ ; producing first spore approximately 175  $\mu$  from base. Proliferation of spore associated with conspicuous local thickening of peripheral wall resembling ring or band; these thickenings occurring in series, giving upper portions of sporophore a more or less moniliform contour.

Spores dark olivaceous; ellipsoidal or short cylindrical with hemispherical ends, sometimes tapering more or less toward basal end; regularly 2 to 3 septate, the septa not associated with constrictions in the peripheral wall; the latter unusually thick, although somewhat thinner at the distal end, and at the basal end diminishing markedly in thickness toward the hilum; the hilum not projecting beyond contour of proximal end. Measuring 15 to 21 by 35 to 50  $\mu$ ; germinating by the production of one or two germ tubes at positions adjacent to or in close proximity to hilum.

Habitat.—Collected during July and August, 1920, near Port Washington, Mineola, Valley Stream, Rosedale, and Douglaston, N. Y.

**HELMINTHOSPORIUM VAGANS, N. SP.**

In July, 1919, shortly after the present studies were begun, the writer's attention was called to a leaf spot of Kentucky bluegrass (*Poa pratensis* L.) that seemed to be generally prevalent in the fields and lawns about Madison, Wis. Although almost invariably present wherever the host was found, the spots were by no means abundant. Usually only a small proportion of the leaves were found diseased, and the majority of these bore evidence of only a single infection. Although no spores were found associated with the spots during this season, the fungus that was obtained in pure culture from the diseased tissue was so similar to other species of *Helminthosporium* in general growth characteristics that observations were resumed the following year.

The leaf spot reappeared late in May, 1920, quite as generally and sparingly as during the preceding season. On June 7, material was collected that showed, moreover, an extension of the trouble to the leaf sheaths, especially toward the base of the plant, the lowermost ones being largely involved in diffuse brown discoloration. The condition thus brought about appeared not unlike that present in wheat plants affected with the disease attributable to *Helminthosporium sativum* that has in recent years become known as "footrot." Much more severely diseased specimens (Pl. 15, A) were collected in Brooklyn, N. Y., on August 13, 1920. The foliar lesions, which are of a bluish-black color quite intense in the center and fading out gradually at the margins, here measured up to 8 mm. in length and 3 mm. in width, although usually not exceeding a half of these dimensions. Some of the leaves had withered prematurely, the withering beginning at the tip and proceeding toward the base. The bases of the lowermost leaf sheaths surrounding the "foot" or "crown" of the plant, were thoroughly permeated with a brown pigment. On microscopic examination it was found that the withered portions of the leaves, as well as the older, dead, discolored sheaths, bore in moderate profusion sporophores with spores typical of the genus *Helminthosporium*.

In addition to the localities already mentioned, the fungus has been collected at many stations in the western half of Long Island, as well as

at Bloomington, Ill.; Annapolis, Md.; Washington, D. C.; Hyde Park, Mass.; Meriden, Conn.; and Lisbon Falls, Me. It thus appears to be quite widely distributed through the northeastern and middle western sections of the country. Indeed, the possibility of a distribution nearly, if not quite, approaching in extent the range of the host, is not to be excluded; for although *Poa pratensis* is one of the most common and valuable of the grasses, both in this country and in Europe, and might thus be expected to be kept under observation by pathologists, the fungus is nevertheless very apt to escape detection. Not only are the foliar lesions caused by the parasite usually small and infrequent, but to a casual observer they may readily be mistaken, in spite of the unbroken epidermis and more intense dark discoloration, for old sori of *Puccinia poarum* Niels., almost always present in some abundance. It need hardly be mentioned that the economic loss caused by a disease ordinarily so lacking in severity is relatively insignificant; yet under certain conditions of moisture and of temperature such as would favor a multiplication of foliar lesions and accentuate the footrot symptoms, the damage may not be altogether unappreciable.

On microscopic examination, the fungus shows considerable similarity to *Helminthosporium sativum*, not only in its effect on the host, but to some extent also in regard to morphological features. The sporophores (Pl. 15, Da-g) found on leaves of *Poa pratensis*, to be sure, are appreciably greater in diameter and frequently greater in length (Pl. 15, Da) than the corresponding structure of the parasite causing spotblotch; and the tendency toward branching exhibited by them (Pl. 15, Db, e) is rarely to be found in *H. sativum*. On the other hand, complete agreement prevails with reference to coloration of the spores, the latter (Pl. 15, Ba-q) when fully mature, being uniformly dark olivaceous. In both species, too, the peripheral spore wall is relatively thick and the number of septa rarely exceeds the usual maximum of 10. With respect to spore shape, the form on Kentucky bluegrass, however, is noticeably different, its conidia being typically straight, never, as a rule, becoming distinctly curved.

The most distinctive character, however, is to be found in the germination of the spore, the germ tubes being proliferated not alone from the end segments, but indiscriminately from both intermediate and end segments regardless of position; and not infrequently every segment participates in the process. (Pl. 15, Ca, b.) This behavior would seem to suggest a measure of affinity with species having straight cylindrical spores like *H. teres* and *H. gramineum*, a suggestion borne out in a measure by the cultural characters of the fungus on ordinary media. Sporulation very rarely occurs on potato dextrose agar, although an abundance of dark aerial mycelium usually is produced. The imbedded mycelium presents an unusually distinctive aspect, for instead of consisting like that of most species of *Helminthosporium*, of a miscellaneous growth of hyphae, it is composed largely of a relatively small number of ramifying systems, all of an intense bluish black color, and bearing hundreds of branching elements.

The literature, beyond a brief abstract published by the writer (31) appears to contain no reference to any species of *Helminthosporium* parasitic on *Poa pratensis*. Karsten (74), in 1884, cited *H. flexuosum* Corda (= *Brachysporium flexuosum* [Corda] Sacc.) as occurring on the leaves of a congeneric host, *Poa stricta*. However, the obvious disparity in size and septation between the spores of the species figured by Corda



(25) and the one found parasitic on Kentucky bluegrass is such as to make it appear altogether improbable that Karsten was dealing with the form under consideration. *Napicladium gramineum* described by I'eck (1808) as destructive to *Poa trivialis* L., is similarly a fungus of much smaller dimensions, the 1 to 3 septate, clavate spores measuring only 10 to 12 by 30 to 60  $\mu$ . In more recent years, Baudyš (9) has reported from Bohemia, a new species of *Helminthosporium* on the living leaves of *Poa trivialis*, which he named *H. poae*. Unfortunately, the writer has not been able to secure a copy of Baudyš' paper and consequently has not been able to decide definitely as to any possible identity of *H. poae* Baudyš, either with the species of *Napicladium* described by Peck, on the one hand, or, on the other, with the fungus attacking Kentucky bluegrass.<sup>10</sup> As *Poa pratensis* is presumably common in Bohemia, Baudyš' failure to record his fungus as developing on this grass would seem to have considerable significance in this connection. The species parasitic on Kentucky bluegrass is accordingly described as new; and because of its widespread occurrence in meagre quantity, the name *H. vagans* is proposed.

#### DIAGNOSIS

##### *Helminthosporium vagans*, n. sp.

Producing well-defined, bluish-black spots 0.5 to 3 mm. wide, 1 to 8 mm. long, on the leaf blades of *Poa pratensis* L.; on the sheaths the spots less definitely circumscribed and near the base of the plant often merging into a generally diffused brownish discoloration.

Conidiophores emerging from stomata or between epidermal cells of tissues some time after death, usually singly or less frequently in pairs; typically simple although occasionally branching; dark olivaceous; usually measuring 8 to 10  $\mu$  in diameter and 50 to 280  $\mu$  in length; 1 to 10 septate, the septa occurring at intervals of 15 to 40  $\mu$ ; approximately straight up to point of attachment of first spore 40 to 150  $\mu$  from base; successive spores produced at apices of moderate or often pronounced geniculations.

Conidia dark olivaceous when fully mature; cylindrical or slightly tapering toward the hemispherical ends; measuring usually 17 to 23  $\mu$  in diameter by 25 to 130  $\mu$  in length; 1 to 10 (usually 5 to 8) septate, the septa not associated with constrictions in the peripheral wall; the latter always thick and including the dark hilum within its contour. Germinating by the production of 3 to 11 germ tubes indiscriminately from end and middle segments, a single germ tube usually being produced from several or all segments.

Habitat.—Collected on *Poa pratensis* at Madison, Wis.; Brooklyn, N. Y.; Bloomington, Ill.; Annapolis, Md.; Washington, D. C.; Hyde Park, Mass.; Meriden, Conn.; and Lisbon Falls, Me.

#### HELMINTHOSPORIUM RAVENELII CURTIS

*Helminthosporium hoffmanni* B. Mss. or *H. hoffmanni* B. & C. 1857, in Introduction to cryptogamic botany, p. 298.

*Helminthosporium tonkinense* Karst. & Roum. 1890, in Rev. Mycol. ann. 12, no. 46, p. 78.

*Helminthosporium crustaceum* P. Hennings 1902, in Hedwigia, Bd. 41, p. 147.

Although of very little economic importance, *Helminthosporium ravenelii*, owing to its conspicuousness and wide occurrence throughout many of the warmer regions of the globe, has become one of the best known members of the genus. It was described in 1848 by Curtis (26), who noted also its abundant distribution in North and South Carolina. This brief account, however, seems to have escaped the attention of mycologists generally; for when Berkeley (11) some years later figured

<sup>10</sup> Since this text was written, Baudyš' paper has become accessible. The Bohemian fungus produces spores provided with 2 to 6 cross-walls and measuring 36 to 73  $\mu$  in length. The foliar spots occasioned by it are described as yellowish with a dark brown margin. In respect to these characteristics, the departures from the morphology of the American parasite would appear to preclude any likelihood of the two forms being identical.

the fungus in his "Introduction to Cryptogamic Botany," he designated it as *H. hoffmanni* B. and C., without any further comment beyond the words, "From specimen on *Sporobolus indicus*. Sent by Rev. M. A. Curtis." Later in a brief descriptive discussion in the "Notices of North American Fungi" Berkeley (12) cited *H. hoffmanni* B. Mss. as a synonym of *H. ravenelii*, which binomial he correctly credited to Curtis alone. Nevertheless, as the publication of the original specific diagnosis in an American journal has apparently remained relatively unknown both in this country and abroad, the name frequently has been improperly attributed to the joint authorship of Berkeley and Curtis.

The earliest collections of the fungus made by Curtis were from North and South Carolina. Material from the rest of the South Atlantic States and from the Gulf States, Georgia, Florida, Alabama, Mississippi, Louisiana, and Texas, as well as from Mexico and China, is included in numerous collections in the herbarium of the Office of Pathological Collections. Specimens of the parasite collected in Costa Rica were distributed by Sydow.<sup>11</sup> Kabát and Bubák<sup>12</sup> distributed material from Uruguay and Theissen<sup>13</sup> material from Brazil. The fungus, moreover, has been reported from Cuba by Berkeley (12), from Bermuda by Seaver (132), from New Zealand by Kirk (78), from New South Wales by Cobb (22), and from the Philippines by Hennings (60), Baker (5), H. and P. Sydow (148), and Yates (160). There is good reason to believe that its distribution is practically coterminous with that of its host, *Sporobolus indicus* R. Whether other grasses also are subject to attack is not altogether certain, although Yates (160) records the occurrence of the fungus on the inflorescence of *Panicum auritum* in the Philippines, and *Fimbristylis* is given as the host on the covers of Theissen's Brazilian specimens. More information concerning the identity of the host material on which these records are based would be desirable. It may be stated that *Sporobolus angustus* Buckl. and *S. elongatus* R., sometimes mentioned as hosts, are listed in the Index Kewensis as synonyms of *S. indicus*.

*Helminthosporium ravenelii* attacks the inflorescence of *Sporobolus indicus* in our southeastern states with such regularity that, as has been observed by Curtis (26), Jennings (67), and others, it is often quite difficult to obtain a specimen of this species of grass entirely free from the fungus. Indeed, there is reason to believe that the distinctive color and texture of the diseased panicle is popularly regarded as commonplace attributes of the host, as is evidenced by the common names applied to it in the United States, namely, "black seed grass" (26) and "smut grass" (47). These terms are fairly accurately descriptive of the later stages, when the infected inflorescence has a black crusted appearance; but much less accurately descriptive of the earlier stages (Pl. 16, A) when the fungus is present as a velvety or spongy layer of a brownish olive color, that only later becomes increasingly dark.

This velvety layer, under the microscope, is seen to consist of crowded sporophores (Pl. 16, B) arising from a mat of interwoven colorless mycelial hyphae that occupy the superficial layers of the affected floral parts. Unlike the homologous structures of nearly all congeneric forms parasitic on grasses, the sporophores of *H. ravenelii* exhibit a constant

<sup>11</sup> SYDOW, H. FUNGI EXOTICA EXSICCATI. *Helminthosporium ravenelii* B. and C. No. 442. 1912.

<sup>12</sup> KABÁT ET BUBÁK. FUNGI IMPERFECTI EXSICCATI. No. 540. *Helminthosporium ravenelii* Curt. et Burk.

1907.

<sup>13</sup> THEISSEN, F. DECADES FUNGORUM BRASILIENSUM. No. 277. 1905.

tendency toward branching. They are light fuliginous to light yellowish in color; very noticeably torulose, hence decidedly variable in diameter, this dimension ranging from 5 to 10  $\mu$ , while the length often exceeds 500  $\mu$ ; and usually septate at intervals of from 20 to 40  $\mu$ .

The spores (Pl. 16, Ca-q), borne in great abundance at the apices and geniculations of the distal portions of the fructifications, are straight or show a slight crescentic or sigmoid curve; rounded at both ends, the apical end often more broadly than the basal end, owing to a tendency toward tapering in the basal and juxtabasal segments; 12 to 19  $\mu$  in diameter by 22 to 78  $\mu$  in length; and 1 to 5 (usually 3 or 4) septate, the septa rarely associated with perceptible constrictions in the contour of the thin peripheral wall within which, at the point of attachment, the small dark hilum is readily observed. The conidia germinate readily in water, sending out a germ tube from one, or more usually from both, end cells. (Pl. 16, Da-e.)

Karsten and Roumeguère (75) have described a fungus from Tonking growing apparently on the same host (*Sporobolus tenacissimus*, the host name given, being listed as a synonym of *S. indicus* in the Index Kewensis) and corresponding to *Helminthosporium ravenelii* in all details relating to habit, color, and structure of conidiophores, as well as to color, structure, and dimensions of conidia. This form, which they designated as a new species, *H. tonkinense*, and regarded as being related to *H. ravenelii*, is undoubtedly altogether identical with the latter. Nor can there be much question that the same holds true also of *H. crustaceum* described from Java by Hennings (58) as forming dark crustaceous effuse tufts on the inflorescence of a species of *Sporobolus*. According to this botanist, *H. crustaceum* is related to *H. ravenelii* but distinct on account of its conidia. Inasmuch as the latter are characterized as—

Oblongae clavatis vel fusoidis, utrinque obtusis, rectis vel curvulis, 40-60  $\times$  12-16  $\mu$ , 3-5 septatis, laud constrictis, fuscis—

in all of which particulars the agreement with *H. ravenelii* is at least reasonably close, the writer is inclined to believe that Hennings's binomial should be regarded as a synonym until some evidence for the justification of a new species has been adduced.

#### HELMINTHOSPORIUM SATIVUM P. K. & B.

*Helminthosporium acrothecioides* Lindfors 1918, in Svensk Bot. Tidskr. v. 12, p. 227.

*Helminthosporium gramineum* of E. C. Johnson, Massee, Palm, Bassi, not Rabenhorst.

*Helminthosporium inconspicuum* of Peck, Atkinson, not Cooke & Ellis.

*Helminthosporium Sorokinianum* Sacc. 1891, in Ztschr. Pflanzenkr., Bd. 1, p. 236-239.

*Helminthosporium teres* of Bakke, not Saccardo.

*Helminthosporium* sp. of Beckwith, Bolley, Evans, Hamblin, Hungerford, McKinney, Stakman, Stevens, Waterhouse.

Although the fungus to which it has appeared advisable to apply the binomial given by Pammel, King, and Bakke (104), probably is the species most frequently encountered by plant pathologists, it has been the subject of much confusion in the literature. This condition is largely attributable to the fact not hitherto generally recognized that it occurs on a number of graminaceous hosts, several of which, moreover, are affected by one or more congeneric parasites. As the specific characteristics of the latter have not always been clearly distinguished, and as the fungus under consideration shows a tendency toward variation in response to varied environmental conditions, occasion for erroneous identification has not been lacking.

## NOMENCLATURE

In 1891, a brief anonymous account (136) appeared concerning *Helminthosporium Sorokinianum*, Sacc. (in litt), a fungus which Sorokin (136) had found occurring on the spikes of wheat and rye in the South Ussurian region in Russia. The spores were later described in Saccardo's (128, v. 10, p. 415-416) diagnosis of the species as—

acrogenis, ovato-fusoides, majusculis, 80-100 x 30, rufobrunneis, 5-10 septatis, passim lenissime constrictis, rectis curvuliscis.

This characterization applies fairly well to the spores found occurring on wheat spikes in the United States with reference to shape and septation. The dimensions given also are not outside of the ranges in dimensions found in this country, the figures given for length corresponding closely enough, although 30  $\mu$  constitutes a maximum width (pl. 18, Fe) not attained by a large proportion of conidia. It appears quite probable, therefore, that the Russian fungus is identical with the American form. However, because the spores of the latter, when fully matured and in a living condition, are dark brown or olivaceous rather than reddish brown, the identity of the two forms can not be regarded as firmly established. Therefore, in spite of the priority of Saccardo's binomial, the writer believes it advisable to treat it as a probable synonym.

In 1909, Pammel (103) recorded the occurrence during the preceding season of a barley disease in Iowa differing from the stripe disease. A more complete account of this trouble was published in 1910 by Pammel, King, and Bakke (104), in which the casual organism was described as having fasciated fuscous brown septate conidiophores, 8 to 10  $\mu$  wide, bearing a large cylindrical dark brown spore, with 7 to 12 divisions, and measuring 15 to 20 by 105 to 130  $\mu$ . The fungus was regarded as closely related morphologically to *Helminthosporium teres*, but in the absence of comparative cultural studies was provisionally given a new specific name, *sativum*. Later, however, one of the authors, Bakke (6), presumably as a result of cultural experiments and in conformity with opinion secured from Saccardo and Ravn, definitely referred the disease to *H. teres* Sacc. Although indications are not wanting that Bakke in this later work was dealing to some extent with the latter organism, the figures as well as the text leave no room for doubt that he was in the main concerned with the same disease and the same parasite that had been discussed in the preceding Iowa publication. Inasmuch as *H. teres* and the fungus causing "late blight" of barley are not identical, representing, indeed, two quite distinct congeneric types, it would seem that Bakke was in error in repudiating *H. sativum* as an independent binomial.

In 1918, Lindfors (84) described from Sweden as *Helminthosporium acrothecioides* a fungus he had discovered on barley seed that had been germinated on filter paper. Its morphological features correspond completely with those of the American fungus developing under the warm, damp conditions obtaining in germination apparatus, when, as the writer has observed in hundreds of instances, discolored barley seeds or wheat seeds affected with "black point" are incubated on moist filter paper. The figures and the characterization of the conidia as "narrow ellipsoid to spindle-shaped, with blunt ends, 60 to 95 by 20 to 24  $\mu$ , with 7 to 9 septa, and a thick, dark olive brown epispore," apply so well to the American form that Lindfors's binomial may very safely be regarded as a synonym.

## OCCURRENCE OF THE FUNGUS ON BARLEY

Of the graminicolous species of *Helminthosporium* thriving in our northern latitudes, *H. sativum* shows, perhaps, the strongest omnivorous tendency, being vigorously parasitic on a number of grasses and occurring on others apparently in a manner more nearly suggesting a saprophytic relation. Its greatest luxuriance is attained, nevertheless, on the foliage of barley, from which it originally was described. According to the records of the Plant Disease Survey, the fungus has been reported on barley in 24 states, including all of the more important barley-growing sections of the country. Güssow (48) early recorded its occurrence on the same crop in Canada.

Although the host is susceptible at any stage of development, the use of affected seed often resulting in the appearance of severe lesions in the basal portions of the young seedlings, the disease usually does not begin to show up in quantity until the plants are heading out. It is manifested by the appearance of spots varying from 0.5 to 3 mm. in width and from 2 to 15 mm. in length, usually dark brown in the center and fading gradually at the margins into the green of the surrounding tissue. (Pl. 17, B.) The lower leaf blades are first affected, the discolored areas multiplying until scores of them may be present on one foliar organ and a considerable portion of the leaf tissue is involved (pl. 17, A). As a result the leaf soon withers and dies, the discolored areas fading slightly and becoming vaguer in outline, while the parts not visibly altered in appearance take on a grayish hue. The foliar spots never become bleached in the center as those caused by *H. leersii* on *Leersia virginica*; nor exhibit a reticulate pattern, like those induced by *H. tere* on barley; nor are they surrounded by a zone of leaf tissue from which the chlorophyll has disappeared like those produced by *H. bromi* on *Bromus inermis*. At the same time the destruction of the lower leaves takes place, the disease makes progress in the younger foliage, which then succumbs in the same way, until the uppermost leaf is affected. The effect of this virtual defoliation is to hasten the ripening processes. In a season favorable for the development of the disease, the grain may be ready for harvesting perhaps two weeks earlier than when the trouble is absent. As might be expected, the yield is decreased in a measure approximately corresponding to the shortening of the growing period.

Although the foliage of barley is most severely attacked the inflorescence, as Pammel, King, and Bakke (104) pointed out, is not immune. The fungus may be found on the glumes and not infrequently on the seed. The diseased kernels usually are readily distinguished because of the dark-brown discoloration at the germ ends, a feature that has, indeed, been utilized by Atanasoff and Johnson (3) in selecting infected material for experiments on the dry-heat treatment. When such discolored kernels are placed in a germinator the fungus becomes visible usually within 24 hours as a delicate white velvety outgrowth, that soon spreads on the filter paper or other material as an effused arachnoid mycelium and produces, if the conditions are not too moist, an abundance of fructifications. A reduction in viability, usually apparent in slightly discolored seed, may become very considerable when the seed is more severely affected. After the basal sheath has been developed this organ often is attacked by the fungus and as a result takes on a yellow or light yellowish-brown discoloration. A considerable proportion of the rootlets

may be invaded in the same way, often stunting their development and softening the discolored cortical tissue. Undoubtedly, quite similar pathological processes take place when the seedlings are planted in soil. The effect of such early infection, moreover, then becomes apparent in the development of the seedling, for, in addition to the lesions on seed, sheath, and root system, conspicuous dark brown spots may occur on the first few leaves, thus giving rise to the thoroughly diseased condition described and illustrated by Atanasoff and Johnson.

It may be mentioned in this connection that a discoloration of barley seed evidently quite similar to that frequently observed in the United States was noted by a number of European writers. Zöbl (163, 164), in 1892, published some papers on "brown-pointed" (*braunspitzige*) barley, in which the discoloration is described as being most intense at the base of the seed and decreasing toward the apex. Although various fungi belonging to the genera *Sporodesmium*, *Cladosporium*, *Helminthosporium*, and *Dematium* were found associated with discolored seeds, Zöbl attributed the brown-point condition primarily to *Cladosporium herbarum*. Puchner (113), in some studies on "black-pointed" (*schwarzspitzige*) barley seed, found that these germinated abnormally, often producing plants the leaves of which bore brown spots. However, when barley seed similarly affected was treated with copper sulphate preliminary to sowing the foliar lesions failed to appear in the seedling, although during the later stages in development the foliage and inflorescence of the originally healthy plant were as subject to attack as those of a diseased specimen. Ravn (115) found a fairly close correlation existing between the prevalence of net-blotch and "brown point" (*brune Spidsers*) in the seed. Without regarding a causal relation as firmly established, he nevertheless appears to have been inclined to see in *Helminthosporium teres* the most probable cause of seed discoloration.

While the evidence adduced by Ravn would appear altogether sufficient to justify his view, as far as conditions in Denmark at the time his investigations were carried on were concerned, the fact remains that in our North Central States *H. teres* is not generally associated with black-pointed barley seeds. On the other hand, as has been mentioned before, the association of *H. sativum* with this condition appears unusually constant. For example, when discolored barley seeds from stock grown in Wisconsin are plated on agar, after proper surface sterilization, only a very small proportion of seeds will fail to give rise to mycelia and conidiophores of the parasite causing spot-blotch. Presumably *H. sativum* is of less frequent occurrence on barley in Europe than in the United States. That it is more common than the absence of references from European literature might lead one to infer is suggested, for example, by Massee's (90) account of *H. gramineum*. The ambiguity with regard to the host range of the fungus, and the longevity, color, and size of the conidia, fortunately is explained by figures of these bodies (fig. 132-6), which show beyond any doubt that this author was dealing not with the stripe fungus but with the parasite causing spot-blotch. In Lindfors' (84) description of *H. acrothecioides* the existence of a species of *Helminthosporium* on barley other than *H. gramineum* and *H. teres* eventually was recorded, but its relation to pathological lesions in the growing plants of either barley or of wheat or rye has apparently not yet been recognized.

## OCCURRENCE ON WHEAT AND RYE

Helminthosporium disease of wheat affecting seedlings as well as older plants was reported, according to the files of the Plant-Disease Survey, as doing considerable damage in several seasons during the past decades, especially in North Dakota and Minnesota. In the former State, Bolley (14) found one or more species of Helminthosporium responsible in large measure for the deterioration of wheat production. Isolations made from various parts of diseased wheat plants revealed the presence, in addition to representatives of the genera *Alternaria*, *Colletotrichum*, *Fusarium*, and *Macrosporium*, of strains of Helminthosporium in the nodes and internodes of the stem, as well as on the surface and in the interior of the kernels (15). Of special interest is the account given by the same author of a type of infection designated as "brown spot" and manifested by brown discoloration of the lower portion of the wheat stems near the soil line. Such attack was found to occasion reduction in tillering, as affected stools usually consisted of only one, or more frequently, two tillers, the other tillers being represented by abortive shoots or intercepted buds. Beckwith's (10) study of the occurrence of soil fungi in North Dakota showed that strains of Helminthosporium were found occurring considerably more abundantly on the nodes and internodes of wheat in the wet season of 1909 than in the dry season of 1910.

Later, E. C. Johnson (71) published the results of experiments on certain cereals with a fungus he designated as *Helminthosporium gramineum* Rabh. Inoculation of young seedlings of wheat, barley, oats, and rye with spores from pure cultures originally isolated from the lower parts of the culms of wheat, as well as from wheat leaves and barley leaves, resulted in the prompt appearances of leaf spot on all the four graminaceous species. When wheat seeds inoculated with spores were sown, their germination was considerably reduced, and the resulting plants were stunted in comparison with uninoculated controls. Indeed, some of the inoculated seeds were attacked so promptly that they had no opportunity to germinate; in other cases, the young plants were killed before they were an inch high. The attack on the surviving seedlings was manifested by a brown discoloration at the base of the culms, usually occurring in the basal leaf sheath, and subsequently extending to the root crown, as well as by the partial brownish discoloration and reduced development of the root system. Barley and oat seed similarly treated were not perceptibly reduced in viability, although the resulting barley seedlings were somewhat retarded in growth and exhibited in smaller degree the same type of discoloration as the diseased wheat seedlings.

In spite of E. C. Johnson's statement that the strain of Helminthosporium discussed in his paper corresponded in cultural and morphological characteristics to the descriptions of Ravn (115), there are strong reasons for suspecting that his fungus was not identical with the parasite causing barley stripe. The latter, in the writer's experience, can not be made to sporulate in pure culture except in meager quantity, any profuse sporulation, such as was presumably induced in all the imperfect fungi employed by E. C. Johnson, certainly never having been observed in *H. gramineum*. Moreover, the pathological lesions produced in his experimental plants obviously bore little resemblance to those of the systemic trouble described by Ravn. On the other hand, the symptoms

correspond accurately with those noted later by Atanasoff and Johnson (3) on seedlings grown from seed infected with *H. sativum*.

In 1918, Palm (101) reported from Java the occurrence of a species of *Helminthosporium* on wheat which he designated as *Helminthosporium gramineum* ([Rabh.] Eriks.). The fungus was found present on the glumes as well as on the perceptibly shrunken kernels, through the agency of which the author believed the infection to be transmitted. The statements relating to the long worm-shaped, usually curved, 6- to 10-septate spores measuring 65 to 110  $\mu$  in length, and 15 to 20  $\mu$  in width, and more especially the accompanying figures, indicate that the Javan fungus differs conspicuously from the parasite causing stripe but shows complete similarity to *H. sativum*, as it occurs on the same host in this country.

Considerable attention has been given during recent years to a diseased condition found prevalent on wheat in the vicinity of Granite City, Ill. Stevens (140) recognized the trouble as a typical footrot which he attributed to a species of *Helminthosporium* found constantly associated with it and producing luxuriant growth on a variety of substrata. The conidia were described as structures approaching a narrow or broadly elliptical shape measuring 24 to 122  $\mu$ , usually 80 to 90 $\mu$ , in length; containing from 0 to 13, usually 5 to 10, septa or false septa; possessing an outer thin dark wall and an inner colorless thick wall; and germinating by the production of one or two polar germ tubes. The causal relation of the fungus with the disease presumably was established, moreover, by successful inoculation of the unwounded internodes of wheat seedlings. When wheat seeds were inoculated with spores of the fungus and germinated in a seed tester, the host tissue was quickly invaded, leading to the production of brownish spots, and under favorable conditions to general rotting and death of the innermost leaves (141). Inoculation of the roots was followed by invasion and discoloration of the cortex.

McKinney (86), who also carried on studies near Granite City, Ill., similarly found a species of *Helminthosporium* present on wheat, associated with lesions often developing during the later stages of the disease. Apparently, however, he did not regard the latter primarily as a foot rot, but rather as a trouble having somewhat different symptoms which, at least during the early spring stages, was not found constantly associated either with any perceptible lesions or with any specific organisms whatever. In a later note, McKinney (87) states that all the strains of *Helminthosporium* isolated from wheat appear to be similar, if not identical, not only to one another, but also to *H. sativum* derived from barley leaves affected with spotblotch. And this similarity was found to obtain with reference to the morphology of the fungi from the two sources as well as to their pathological properties as evidenced in cross inoculation experiments.

A critical study of a *Helminthosporium* disease of wheat and rye was published recently by L. J. Stakman (138). The malady, which was unusually common in Minnesota in 1919, was manifested early in the season as a seedling blight, characterized by brown discoloration of the roots, either in extensive patches or in numerous small irregular lesions; by the presence of rust-brown streaks or blotches on the "foot" or base of the stem, later progressing to the inner sheaths; and by a general dwarfing of the plants, the leaves of which were conspicuously stunted, very narrow and pale reddish tan in color. Many of the affected plants



died in the seedling stage. Others, however, recovered and grew to maturity, although the older plants frequently suffered not only from the persisting foot lesions, but also from secondary infections. As a result of the latter, numerous dark brown spots, about 1 mm. long, appeared on leaves, nodes, neck, and glumes, and brownish streaks were found present on the internodes. The discolored areas later became paler, and developed a coating of *Helminthosporium* fructifications. On potato dextrose agar the fungus produced an abundance of spores, which were described as being straight or curved, dark blue to brown in color, 3- to 8-septate, and measuring on the average 41 by 20  $\mu$ . Cross inoculations carried out with cultures of the *Helminthosporium* species isolated from wheat and rye, indicated that the fungi from these two different hosts were as indistinguishable in respect to pathogenic properties as in respect to morphology. It is especially significant that the strains from rye infected barley, and that a form identified as *Helminthosporium sativum* produced infection on wheat.

A form of disease strikingly similar to that described by L. J. Stakman was observed on wheat by Bassi (8) during the season of 1921 near Piacenze in northern Italy. Two types of the malady, "nerume," were recognized, one affecting the tender shoots of wheat or rye in autumn, the other present the following summer on more mature plants. In the former type, growth is conspicuously stunted, the roots and nodes are involved in decay, and the leaves, besides being greatly reduced in size, assume a light reddish, olivaceous color and eventually wither and die. Characteristic lesions are found on the base of the culms either as local dark reddish streaks or as a more extensive discoloration. The roots are attacked also, the cortical tissue often being injured so severely that when the plants are pulled up the vascular elements are extracted from the cortex which remains behind. The second type of infection is manifested by the appearance on the leaves of numerous small dark brown spots, but more particularly by an attack on the nodes ("marciume dei nodi") so that the latter frequently show on their margins dark brown horizontal lines composed of spores of the fungus. Brown lesions occur on the internodes, as well as on the glumes, which in the final stages, after sporulating commences, assume a dark tinge. The kernels also often are attacked, then becoming discolored and failing to attain full development. The fungus on specimens of diseased plants sent to Padua for examination was identified as *Helminthosporium gramineum*.

In a recent paper Hamblin (49) reports the prevalence of a footrot disease of wheat due to a species of *Helminthosporium* in widely separated regions of New South Wales. The damage occasioned by the fungus in 1921 is believed to have been greater than the loss due to "take-all," varying from 2 to 3 up to 85 and 90 per cent. Apparently the symptoms of the Australian trouble are very similar to those noted in the United States and Italy. Tillering is greatly reduced, the tillers being usually not in excess of two or three. As the root system is poorly developed and affected by decay, diseased plants are easily pulled up. At and below the ground level the base of the plant shows brownish discoloration, either uniformly diffused through the culm and sheaths or present in the form of spots or streaks. The inflorescence of affected plants fails to attain normal development, some of the heads failing to set any seed, others setting seed only in a portion of the spikelets, and still others setting seed in all the spikelets, but the seeds remaining pinched and undersized. Although Hamblin gives no textual descrip-

tion of the parasite, the figures of the sporophore and conidia show an unmistakable resemblance to those of *H. sativum* developing in artificial culture or on natural substrata under moist conditions.

Another reference to the relation of an unidentified species of *Helminthosporium* to root rot of wheat is given by Raeder (114) who observed considerable damage caused by this trouble in Idaho. His description of the symptoms—stunting of the heads, shriveling of the grains, discoloration of the sheath at the base, and occasionally also of the lower nodes—certainly suggest the spotblotch fungus. Snowden's (135) report of *H. sorokinianum* on wheat in Uganda may plausibly be interpreted as referring to the same parasite.

The accounts briefly summarized in the preceding paragraphs concerning the occurrence of a species of *Helminthosporium* on wheat in association with well-defined lesions raise the questions as to whether one or several congeneric forms are concerned. The writer has examined scores of strains of *Helminthosporium* isolated from black-pointed wheat seeds (Pl. 18, Aa-Cb), from discolored portions of the stems and leaves of both young (Pl. 18, E) and more mature wheat plants, and from conidial fructifications occurring on the glumes of wheat heads (Pl. 18, D). Although the material was obtained from a considerable number of localities in the middle western section of the United States, no constant morphological differences such as distinguish the different species discussed in this paper, could be detected between the various strains. Nor was it possible to recognize any significant differences between the forms isolated from wheat and various strains of *H. sativum* isolated from barley leaves heavily affected by spot blotch. In some preliminary cross inoculation experiments undertaken by the writer, barley and wheat seedlings proved equally susceptible to attack by strains derived from the same and from the reciprocal host; and the lesions produced were indistinguishable regardless of the source of the inoculum. Such identity of morphological and physiological characteristics, altogether in harmony with the findings of L. J. Stakman (138) and of McKinney (87) have convinced the writer that for the most part a single species of *Helminthosporium*, namely, *H. sativum*, is involved in the widespread infection of wheat manifested variously by such symptoms as stunting of growth, seedling blight, basal browning, root rot, foot rot, node decay, leaf spot, stem discoloration, and black point. And as the reports of Sorokin, L. J. Stakman, and Bassi indicate, the same fungus evidently is associated with similar pathological conditions in rye.

In another connection, it is true, attention has been called to the fact that the type of injury occasioned by *Helminthosporium sativum* on the wheat plant is duplicated by the attack of several congeneric species on other hosts, as, for example, *H. vagans* on *Poa pratensis* and *H. monoceras* on *Echinochloa crus-galli*. The occurrence of one or more species of *Helminthosporium* other than *H. sativum*, associated with similar lesions is consequently not to be excluded as a possibility. Nevertheless, there seems to be little reason to believe that *H. gramineum* has ever been found parasitic on wheat in spite of the papers of E. C. Johnson, Palm, and Bassi, indicating such parasitic relation. The reports of Johnson and of Palm, as has been pointed out, appear to have been based on obviously erroneous identification of the fungus concerned. And the form of disease described by Bassi corresponds so well to the trouble investigated by L. J. Stakman that it is hardly probable that two separate species

are involved. While the Minnesota fungus was not definitely identified as *H. sativum*, its similarity to the latter fungus with regard to morphological and cultural characters is certainly very close. To be sure, L. J. Stakman states that the spores of the wheat parasite, while resembling those of *H. sativum* in shape, "contain few septations and are shorter than those described by Pammel, King, and Bakke." However, as the Minnesota fungus appears to have been studied chiefly in pure culture, where the spores of *H. sativum* ordinarily become greatly reduced in length and number of septa as compared with those developed on the host in the field, on which the original diagnosis undoubtedly was based, the difference is very readily explained.

It may not be superfluous to mention that several species of *Helminthosporium*, morphologically altogether different from *H. sativum*, have been reported as occurring on various parts of wheat and rye plants. Thus Palm (101) records the presence on the spikes of this host in Java of a relatively innocuous form with small geniculate spores which he identified as *H. geniculatum* T. & E. (152). Hennings (59) earlier described as a new species, *H. tritici*, another small-spored but apparently different fungus collected by Zimmerman in the region formerly included in German East Africa. The African form, described as being very injurious, is evidently similar in general habit to *H. ravenelii*, developing a dense crusty coating on the culms, leaves, and heads of the host. Stevens (141) makes mention of a geniculate-spored form evidently different from the one usually encountered on diseased wheat in Illinois; while L. J. Stakman records the isolation from the same host of strains of *Helminthosporium* producing smaller spores than the species generally found associated with the Minnesota disease. A species of *Helminthosporium* obviously different from *H. sativum* was described from Alabama growing apparently as a saprophyte on decaying culms of rye by Atkinson (4) under the name of *H. tuberosum*. The same author also reported *H. inconspicuum* "on living and languid leaves of *Secale cereale*," evidently in a parasitic relation. It appears at least not improbable that Atkinson, following Peck (107), applied the binomial of Cooke and Ellis not to *H. turcicum*, but to a form morphologically very similar if not altogether identical to *H. sativum*.

As has been indicated in other connections, when the inflorescence of wheat is attacked (Pl. 18, D), the kernels often are affected, resulting in dark brown, bluish brown, or nearly black discolorations involving more or less extensive and irregular patches at the germ end or extending along the ventral furrow (Pl. 18, Ba-Cb). Hungerford (64) and Waterhouse (156) noted the presence of *Helminthosporium* fructifications on wheat grains. A special type of such pathological effect in which the discoloration is relatively intense and limited to the region occupied by the embryo (Pl. 18, Aa, b) has become known as "black point." It is characteristic especially of durum wheat, having been reported by Bolley (14) in 1910 as being on the increase on this species, and more recently made the subject of investigation by Evans (38) as well as by Weniger (158) and by Henry (61). Greenhouse experiments, now in progress, the results of which will be published later, have shown that, compared to healthy seeds, badly discolored seeds show a much smaller degree of viability, and that the plants resulting from them frequently develop lesions varying in number and severity. (Pl. 18, E.) It may be mentioned that other fungi, notably one or several species of *Alternaria*, also have been found associated with discoloration of wheat grains, which, however,

while somewhat similar to the type of discoloration attributable to *H. sativum*, can usually be distinguished by its lesser intensity, being diffused brownish rather than dark brown.

Especially pertinent in this connection is Henry's (61) recent report of the isolation from black-pointed wheat kernels, of certain forms he designated as "*Helminthosporium* sp." and "*Brachysporium*," which, while of much less frequent occurrence than the spot-blotch fungus, were found to be efficient causes of black point. The writer, who was kindly permitted to examine transfers of these fungi, can only confirm the correctness of Henry's judgment in regarding the "*Helminthosporium* sp." as distinct from *H. sativum*. It appears to be different from any species figured in this paper, though, perhaps, most suggestive of *H. monoceras*. The strains designated as "*Brachysporium*" appear to represent one or several smaller-spored forms that might not improperly also have been referred to the genus *Helminthosporium*, bearing a good deal of resemblance to a species frequently observed by the writer on withered leaves of *Danthonia spicata* (L.) Beauv.

It may not be amiss to refer here to a fungus described under the name *Podosporiella verticillata* by O'Gara (98) who found it on the kernels of germinating wheat in the Salt Lake Valley, causing stunting of the resulting seedlings, and an uneven stand. The conidia of this form show a very obvious general resemblance to those of the larger species of *Helminthosporium*. The synnemata figured by O'Gara are evidently not dissimilar from the threadlike or columnar structures observable, for example, in cultures of *H. inequalis* Shear, or of *H. cyclops*. Indeed, except for the arrangement of the conidia on the sporophore, which according to O'Gara is verticillate, the fungus shows considerable similarity especially to the last named of these species.

While *Helminthosporium sativum* has not been reported as attacking maize in the field, the writer has found it of more or less frequent occurrence on the dead remains of mature plants. Cultures derived from fructifications on old culms and leaves were not observed to differ in any important detail from cultures of the spot-blotch fungus isolated from barley. In a few instances, sporophores and spores of a form similar to *H. sativum*, at least in respect to morphological characteristics, were found on dead areas of leaves of sweet corn collected on Long Island. However, as the fructifications of *H. turcicum* were also present in excessive abundance, it was not possible to draw any definite conclusions concerning the biological relations of the form under consideration. In general, its distribution on corn suggests a saprophytic existence on this host, modified, perhaps, by a capacity to establish itself on moribund foliage. It may be mentioned in this connection that while Stevens found his strains of the Illinois foot-rot organism capable of infecting corn and corn-fodder, L. J. Stakman secured negative results from the inoculation of corn plants with strains of *Helminthosporium* isolated from a node of affected wheat and from rye seed. In some preliminary infection experiments the writer applied spores of *H. sativum* from barley to corn seedlings about 5 inches high, very liberally stroking them in water suspension on the moistened leaves with a spatula, and then confined the seedlings in a saturated atmosphere for 48 hours. At the end of this time the leaves had lost their mechanical rigidity and the entire plants looked as if they had been steamed, although the controls, treated in the same way except that no inoculum was applied, were nearly normal. That much significance attaches to such injury, unlike any naturally

produced by the parasite on any host, and, indeed, unlike any injury produced under conditions obtaining in nature by any congeneric form with the exception of *H. micropus*, seems altogether improbable. The results obtained by exposing experimental plants to the rigorous treatment incident to the application of relatively large quantities of inoculum and incubation in a saturated atmosphere during long periods, should at all events be interpreted with caution. And the necessity of caution would seem especially evident in dealing with a fungus, more or less promiscuous in its parasitism, the experimental host range of which would tend to be out of all proportion to its actual range in nature.

The occurrence of *Helminthosporium sativum* on corn has brought about a measure of confusion regarding the identity of *H. turcicum*. It is interesting to note that in Peck's account (107) of *H. inconspicuum*, the spores of this fungus are described as "nearly black, septate up to 8 to 9 times;" and figured as decidedly dark, curved cylindrical, with rounded ends, attached to sporophores emerging singly from the substratum. In all details of habit and morphology the correspondence with *H. sativum* is much closer than with *H. turcicum*; and it appears probable that although Peck observed the corn disease correctly, he inadvertently based his account of the fungus not on the well characterized parasite, but on the adventitious form. Specimens deposited in the herbarium of the Office of Pathological Collections of old corn leaves from various localities in the United States, labeled "*Helminthosporium inconspicuum*," on examination, were found to bear dark olivaceous spores with non-protuding hila, not dissimilar from those of *H. sativum*, although their collapsed condition precluded the possibility of reliable identification.

#### OCCURRENCE OF FUNGUS ON GRASSES OTHER THAN CEREALS

Bolley (15) reported the occurrence of a species of *Helminthosporium* on quack grass collected in North Dakota and Wisconsin. As the fructifications developing on this host were held responsible in certain instances for the infection of wheat plants in the field, it is apparent that he regarded the species as identical to the parasite causing root rot and brown spot. In any case, the writer found *H. sativum* quite commonly present on quack grass in the northern tier of Middle Western States, being associated with a leaf-spot disease, affecting the foliage of plants of all ages. (Pl. 18, H.) The discolored leaf areas, although quite numerous, are decidedly smaller than those characteristic of spot blotch of barley, occurring usually in the form of linear streaks, dark chocolate brown in color, 0.3 to 0.4 mm. in width and 0.5 to 3 mm. in length. During the season of 1919 the writer collected in the vicinity of Madison, Wis., diseased green leaves as early as May 6, and as late as October 24. The injury to the host appeared to be greater than that resulting from the parasitism of *H. tritici-repentis*, although neither of the two congeneric parasites could be said to have been especially destructive. In the region about New York City, during the season of 1920, the leaf spot due to *H. sativum* was, however, relatively rare, being considerably less abundant than the blight due to *H. tritici-repentis*.

A fungus quite similar to the parasite causing "late blight" of barley was reported by Pammel, King, and Bakke (104) as occurring on *Festuca elatior* L. (= *Festuca pratensis* Hud.) in Iowa. Examination of material deposited in the herbarium of the Office of Pathological Collections only confirms the opinion of these authors concerning the similarity of the lesions to those characteristic of spotblotch of barley, and the very

probable identity of the parasite to *H. sativum*. It is interesting to note, however, that in the vicinity of New York City and Washington, D. C., during the seasons of 1920 and 1921, meadow fescue was not observed affected with the spot blotch described from Iowa, although very commonly showing the symptoms of the net blotch disease discussed elsewhere in this paper.

In addition to the graminaceous species already discussed, the literature contains references to successful infection of other species, by artificial inoculation with strains of *Helminthosporium* that, as has been pointed out, may be referred to *H. sativum*. Thus Massee (90) secured growth and abundant sporulation by transferring spores of the fungus he regarded as *H. gramineum* to leaves of *Festuca ovina*, *Briza media*, *Dactylis glomerata*, *Poa annua*, and *Arrhenatherum avenaceum* that had been cut off and incubated in a damp chamber. Stevens (141) secured infection of Sudan grass and millet as a result of inoculation with the footrot organism. E. C. Johnson (71) found that the strain of *Helminthosporium* isolated by him from the node of a wheat plant seriously attacked the foliage of young oat seedlings. L. J. Stakman (138) reports positive results from the inoculation of over a dozen new hosts with strains originally isolated from wheat and from rye. Taken altogether the results are not in complete harmony, a fact which may probably in part be explained by differences in the conditions under which the experiments were carried on. The method employed by Massee certainly would seem to make for an inordinately extensive experimental host range. On the other hand, the existence of biological races, corresponding to those of stem rust (*Puccinia graminis*) for example, is not outside the realm of possibility. Indeed, absolute agreement in regard to host interrelations between different strains of a fungus attacking so many allied graminaceous species as *H. sativum* could, perhaps, scarcely be expected.

#### MORPHOLOGY OF THE FUNGUS

The fructifications of *Helminthosporium sativum* make their appearance after the death of the affected tissue, emerging from the stomata or more frequently between the epidermal cells singly or in fascicles of 2 or 3. (Pl. 17, Ea-e.) According to Pammel, King, and Bakke (104), the sporophores vary from 8 to 10  $\mu$  in width, but these figures appear somewhat too high, the measurements for this dimension varying usually from 6 to 7  $\mu$ , and rarely exceeding 8  $\mu$ . The first spore is produced generally at a distance of 50 to 90  $\mu$  from the base. The scars marking the points of attachment of successive spores at well-defined geniculations occur at very variable intervals, approaching 5  $\mu$  as a minimum limit, and occasionally exceeding 60  $\mu$ . As found in nature, when sporophores rarely show more than 5 or 6 scars or more than 8 fructa, the distances between the latter usually varying from 5 to 40  $\mu$ . The measurements for *H. teres* given by Bakke (6), 150 to 180  $\mu$  by 60 to 80  $\mu$ , while obviously incorrect for the width of the sporophores of the causal organism of spotblotch or "late blight" of barley, probably as a result of a typographical error, are also sufficiently in excess of the usual range in length of these structures, 110 to 150  $\mu$ , to indicate that this author was, indeed, in this instance referring to the parasite causing netblotch.

Such indication is strengthened by the portions of his description of the spores referring to their length and color, "150 to 130  $\mu$ ," and "pale

greenish gray," respectively. Plate 18, Fg, represents a 12-septate spore of *Helminthosporium sativum* that was scraped from a wheat inflorescence and found to measure  $134\ \mu$  in length, which may be regarded as approximating the maximum for this dimension. The maximum width represented, for example, in the spore shown in Plate 18, Fe, is approximately  $30\ \mu$ . The minimum for width of spore, as found in material occurring in nature, is about  $14\ \mu$  (Pl. 17, Ca), that for length in the region of  $25\ \mu$  (Pl. 18, Fc). When collected on diseased barley (Pl. 17, Ca-i) or quack grass leaves (Pl. 18, Ga-k) in midsummer, the spores are typically slightly or distinctly curved; 3 to 10 septate, widest near the middle, tapering slightly or sometimes quite considerably toward the ends which are rounded off abruptly, and show a hemispherical or hemiellipsoidal contour; and measuring usually 15 to 20 by 60 to  $120\ \mu$ . On wheat heads, (Pl. 18, Fa-q), or on the bases of wheat or barley plants, apparently in response to more moist conditions, the spores are more apt to be atypical, being either straight or, if curved, curved irregularly (Pl. 18, Fd, f, j); showing unusual variability in width, which fluctuates not only with respect to different individuals but also in respect to different portions of the same spore (Pl. 18, Fi); exhibiting often marked irregularity in regard to septation, the septa occurring at unequal intervals, often at planes decidedly oblique to a plane perpendicular to the longitudinal axes of the spore (Pl. 18, Fk, n), and occasionally quite approximating a longitudinal position, thus bringing about a muriformly septate condition (Pl. 18, Ff, h, i). The same departures from the curved, long-ellipsoidal type is exhibited also by spores developed on plants in the greenhouse and perhaps to an even greater extent by those produced in pure culture on artificial media. In the latter case the diminution in size is unusually great, the spores there (Pl. 19, C, Da-c) generally not exceeding in length more than one-half the length of the typical ones to which, however, they are not markedly inferior in width. Very frequently, indeed, they become reduced to subspherical bodies, not appreciably greater in length than in diameter, often nonseptate or with a single cross wall (Pl. 19, C). A straight, short ellipsoidal shape is thus characteristic of the spores developed in the greenhouse or in artificial culture (Pl. 19, E), a shape which may be modified by irregular curvatures or distentions, or by the flattening or even incipient bifurcation of the apical end (Pl. 19, Dc).

But, however variable in shape, the spores of *Helminthosporium sativum*, when fully matured, are uniformly of a dark olivaceous color, and always exhibit a thick peripheral wall and a conspicuous hilum that is situated within the contour of the rounded basal end. As long as the peripheral wall is uninjured, germination regularly proceeds by the proliferation of two terminal germ tubes, one at the apex and the other immediately adjacent to the hilum (Pl. 17, Da, b; Pl. 18, Gc; Pl. 19, of b, d). Atypical spores, with the distal end flattened or bilobed, produce three germ tubes, one arising from each of the lateral apices as well as from the proximal end. (Pl. 19, Dc.) Germ tubes apparently are never produced normally from the intermediate segments. Viability is retained for a considerable period of time, spores from material stored in the laboratory a whole year having been germinated by the writer without much difficulty.

*Helminthosporium sativum* is very readily cultivated on the substrata ordinarily employed in laboratories. On hard potato glucose agar containing an abundance of organic food material the aerial growth is

produce into the protruding hilum; 3 to 10 septate, the septa not usually associated with perceptible constrictions in the peripheral wall. The peripheral wall at black-nick as in *H. sativum* or *H. vagans*, except at the apex and about a sub-narrow zone at the proximal end immediately adjacent to the hilum, where it remains thin. Germinating by the production of two polar germ tubes, one from each of the thin-walled regions.

On artificial media, at ordinary temperatures, producing conidia smaller than those produced under natural conditions but of the same characteristic shape. Vegetative mycelium light fuliginous, 2 to 4 mm. in diameter, anastomosing abundantly by smaller subhyaline branches without the production of lobulate segments. Sporophores fuliginous, thin-walled, often exhibiting a tendency toward branching; approximately 5  $\mu$  in diameter, arising abruptly as branches from vegetative hyphae; or narrow at the point of origin and expanding more gradually; provided with septa generally at intervals of from 30 to 50  $\mu$  and proliferating spores at considerably shorter intervals than in nature, thus producing a moderately compact racemose cluster.

HABITAT.—Collected at Port Washington, N. Y., September 20, 1920, in a moderately moist situation near the sea.

#### HELMINTHOSPORIUM HALODES, N. SP.

During the latter part of the growing season of 1920 the writer kept under observation a stand of *Distichlis spicata* (L.) Greene growing on a salt marsh near Douglaston, N. Y., on the northern coast of Long Island. Collections of the grass made on different dates, September 10, September 26, and October 5, revealed indications of injury immediately suggesting similarity to the symptoms of spotblotch and footrot produced on other graminaceous hosts by various species of *Helminthosporium*, like *H. sativum*, *H. monoceras*, and *H. vagans*. The lesions were present on the otherwise healthy foliar parts as dark, discolored areas, with a bluish cast, not definitely circumscribed, the margins fading insensibly into the green of the surrounding tissue. (Pl. 22, A.) On the leaf blades the discolored spots were generally relatively infrequent; they were found more commonly on the sheath, especially on the upper portion immediately below the attachment of the blade. After the death of the affected tissue, the discoloration usually lost some of its intensity, the spots then appearing as rather vague blotches not readily distinguishable from similar blotches commonly found on the dead plants but attributable to other agencies.

Microscopical examination of dead affected foliar parts revealed a species of *Helminthosporium* present in meager quantity as the probable cause of the disease. Sporophores (pl. 22, Ef-g) occurring on the leaf blades were always found entirely denuded; and whatever spores could be discovered were as frequently found adhering to obviously unaffected tissues or lodged under the upper edge of the leaf sheaths as scattered on the epidermis in proximity to the sporophores. In view of the fact that the host is occasionally inundated by tidal water, an explanation for such a condition is manifestly not difficult to find. To determine whether the disease observed bore any relation to the *Helminthosporium* fructifications, freshly affected green parts were dissected out and incubated in a damp chamber. After 15 days the discolored areas were covered with a dense growth of conidiospores bearing conidia corresponding quite closely to those found in nature. Pure cultures made from spores obtained directly from the material as it was collected and from spores developed in the damp chamber further demonstrated the specific identity of the two lots of material and consequently also the parasitic nature of the fungus originally observed.

As might be expected from the similarity of pathological symptoms, the fungus shows a fairly close resemblance to *Helminthosporium sativum*.



The conidiophores, as occurring in nature (Pl. 22, Ef, g) septate on the natural substratum in a damp chamber (Pl. 22, Ea-e), wheat what inferior in diameter to those found on barley leaves affected by spotblotch and generally noticeably shorter. The conidia are of same type as those of *H. sativum*. As found on material collected in the field (Pl. 22, Ba-f), however, they are usually more narrow and not as regularly crescentic, being more frequently straight or irregularly curved. In color they are usually brownish yellow instead of olivaceous. In a certain proportion of the spores, moreover, the end cells are less deeply colored and the basal and distal septa appear conspicuously darker or heavier than the intermediate cross-walls. (Pl. 22, Bd-f.) When developed in a damp chamber on diseased host material the conidia approach those of *H. sativum* in depth of coloration; but the distinction between dark intermediate segments and subhyaline or fuliginous end segments set off by conspicuously accentuated septa becomes a constant characteristic. (Pl. 22, Da-f.) And while the apical end is rounded off quite abruptly as in *H. sativum*, the proximal end shows a more perceptible tendency to taper, recalling in some instances the decidedly acuminate contour of the proximal portion of the spores of *H. monoceras*. As in the latter species, this gradual tapering is associated with a hilum that protrudes from the basal contour of the conidium.

In the species parasitic on *Distichlis spicata* the peripheral wall of the conidium varies more or less in thickness, depending apparently on the conditions under which the fructifications are developed. The darker, more mature conidia usually possess a peripheral wall which, if not as massive as in *Helminthosporium sativum*, nevertheless is of at least moderate thickness. As in *H. monoceras*, the wall is very thin at the apical end and over a narrow zone at the proximal end immediately adjacent to the hilum. Germination regularly occurs by the production of two polar germ tubes, one from each of these thin-walled regions. (Pl. 22, Ca, e, i.) In the case of immature spores and often in the brownish yellow ones found in nature the peripheral wall is thinner, frequently collapsing when the contents of the segments inclosed degenerate. Such spores may germinate by the production of lateral germ tubes from one or more of the intermediate segments as well as from the end segments (Pl. 22, Cb, c, d, f, g). This mode of germination, however, can scarcely be regarded as typical for the species.

The fungus is cultivated readily on artificial media, producing a luxuriant dark olivaceous aerial growth, consisting of a variable quantity of mycelium bearing an abundance of sporophores. When grown on tap-water agar, the fructifications (Pl. 23, A-C) resemble those developed on parts of the diseased host after incubation in a damp chamber. The spores (Pl. 23, B, D-G), which are attached at short intervals on the very irregular sporophore at wide angles and in moderately compact racemose arrangement, however, are usually perceptibly shorter. As in *Helminthosporium sativum*, the conidia frequently exhibit irregularities in shape, including flattening or bifurcation of the apical portion. (Pl. 23, D, F.)

Besides resembling in some details the different species which have already been mentioned, the fungus suggests comparison with *Helminthosporium leersii*, and perhaps more especially with *H. oryzae* and *H. rostratum*. From *H. leersii* it may be distinguished readily by the subhyaline end segments, the accentuated end septa, and the protruding hila characteristic of its spores. While *H. leersii* on artificial media

produces a greyish aerial mycelium, the growth of the parasite on *Distichlis spicata*, as has been noted previously, has a very dark if not almost black appearance. *Helminthosporium oryzae*, on the other hand, could be confused with the fungus under consideration only when cultivated on a substratum on which its spores develop dark pigment, as, for example, potato dextrose agar. The greater dimensions of the conidiophores and conidia produced by the rice parasite and the position of the hilum within the contour of the peripheral spore wall constitute, however, features by which the two species can be distinguished in spite of some similarity due to the occurrence of subhyaline end segments in both. Moreover, while the spores of *H. oryzae* tend to taper strongly toward the tip, and to a smaller extent toward the base, those of the fungus on *Distichlis spicata* show relatively slight tapering toward the abruptly rounded apex, while the proximal portion usually tapers markedly toward the base. The resemblance between the latter fungus and *H. rostratum* is attributable to similarity in color of the conidia as well as to the presence in the spores of both species, of protruding hila, and of accentuated proximal and distal septa. The parasite on *Distichlis spicata*, however, is inferior in the dimensions of both sporophores and spores and never exhibits the rostrate modification of the apical end characteristic of the conidia of *H. rostratum*. It appears not to have been described hitherto. As it is the first member of the genus, as far as the writer is aware, to be found occurring as a parasite on a host more or less frequently flooded with sea water, the specific name *halodes* is suggested.

#### DIAGNOSIS

#### *Helminthosporium halodes*, n. sp.

Occurring on the foliar organs of *Distichlis spicata* (L.) Greene, on which it causes a disease not usually destructive, manifested by the appearance of poorly defined bluish discolored areas especially on the leaf sheath immediately below its juncture with the blade.

Conidiophores arising from discolored spots after death of host, singly or in groups of two; measuring generally 4 to 7  $\mu$  in diameter by 60 to 150  $\mu$  in length; producing first spore usually 60 to 100  $\mu$  from base, successive spores at intervals of 5 to 15  $\mu$  at apices of geniculate irregularities; 1 to 5 septate, the length of segments highly variable, typically 15 to 30  $\mu$ .

Conidia as produced under natural conditions straight or curved; measuring 10 to 14  $\mu$  in width by 20 to 105  $\mu$  in length; 1 to 12 septate, the septa in immature spores associated with barely perceptible constrictions of the peripheral wall, in mature spores not associated with constrictions; brownish yellow, the end segments sometimes lighter in color or subhyaline, and delimited by accentuated septa; tapering slightly toward the broadly rounded apex, and more markedly toward the more narrowly rounded or somewhat acuminate basal end, which is uniformly distinguished by the protruding hilum. Mature spores germinating typically by the production of two polar germ tubes, produced at thin-walled regions at tip and immediately adjacent to hilum; germination of immature thin-walled conidia often atypical, owing to the production of additional germ tubes from one or more intermediate segments.

When cultivated on natural substratum in damp chamber, fructifications similar but capable of more extensive development; spores somewhat shorter, usually thicker, and tapering more perceptibly toward both ends; dark olivaceous, the end segments subhyaline or light fuliginous and set off by accentuated septa. On artificial media not rich in organic food material conidiophores arising as lateral branches from prostrate hyphae, bearing conidia in moderately dense racemose arrangement at geniculate irregularities occurring at short intervals; conidia as on natural substratum dark olivaceous provided with subhyaline end segments and with moderately thick peripheral wall but shorter, often being nearly ellipsoidal in shape.

**HABITAT.**—Collected at Douglaston, N. Y., near New York City, in September and October, 1920.

## HELMINTHOSPORIUM TURCICUM PASSERINI

*Helminthosporium inconspicuum* Cooke & Ellis 1878, in Grevillea, v. 6, p. 88-89.

The disease of maize commonly known as "leaf blight" and less frequently as "white blast" is found in many regions of the globe in which this important cereal is cultivated. It appears to have been first observed by Passerini (105) in 1876, who noted its occurrence in Italy under the term "nebbia" and attributed it to a species of *Helminthosporium*, which he named *H. turcicum*. Two years later, Cooke and Ellis (24) described a form from New Jersey as *H. inconspicuum*, similarly thriving on maize. Although the descriptions of the American and Italian forms are not especially similar, there appears to be good reason to believe that they were based on material belonging to the same species.

In the account given by Cooke and Ellis, the American fungus is very briefly described:

Tenuissime effusum. Hyphis elongatis, septatis, nodulosis, pallidebrunneis. Sporis lanceolatis, 3-5 septatis; episporio tenui.

On *Zea mays*.

Effused, but so thinly as not to be visible to the naked eye. Spores 0.08 to 0.12 by 0.02 mm., at first with the endochrome divided, at length septate.

The parts of the statement regarding the number of septa, the visibility of the fructifications, and the alleged division of the endochrome previous to the division of the spore can scarcely be regarded as altogether correct, either for the parasite causing leaf blight or for *Helminthosporium sativum*, which, as has been pointed out in another connection, is known to occur on the organs of mature corn plants. The parts regarding the description of the sporophore, the shape and measurements of the spore, as well as the figure of the latter that accompanies the text, on the other hand, might apply about equally well to both species. Indeed the chief reason for regarding the description given by Cooke and Ellis as applying to the leaf blight fungus is not found in the single distinctive characterization—that concerning the thin episporium—but rather in the abundance of the parasite in the general region in which Ellis made his collections. In 1881, Peck (107) recorded the leaf blight disease in New York, although, as has been stated in another connection, his description of the fungus is less applicable to the parasite under consideration than to *H. sativum*. The trouble again was found in New York by Stewart (144) in 1896. In 1889 Thaxter (149) observed a serious outbreak of the disease in Connecticut, where 14 years later Clinton (21) found it again doing considerable damage. In 1903 the malady was quite destructive also in Delaware, according to the account given by C. A. Smith (133). During the same season Stone and Monahan (145) noted its abundance in Massachusetts; and Orton (99) found it very general also in eastern Pennsylvania and New Jersey. Since then it has been reported repeatedly not only from the Middle Atlantic and New England States, but from other sections as well, the records of the Plant Disease Survey indicating its presence in nearly every State east and in some of the States immediately west of the Mississippi River.

Stevenson and Rose (143) reported leaf blight of corn as occurring in Porto Rico. Robinson (122) in 1911 observed the disease in the Philippines, where it has been noted also since then by Baker (5) as well as by Reinking (117). Tryon (153) records an outbreak of the trouble in New South Wales in the season of 1886 so destructive as to attract

widespread attention; and in recent years Darnell-Smith (27) lists "leaf stripe" among the diseases affecting maize in the same Province. Yoshino (161) reported *Helminthosporium turcicum* as occurring on *Zea mays* in the Province of Higo, Japan, in 1905. According to Butler (19) the disease is fairly common in India and occurs in South Africa. The European literature concerning the disease does not appear to be extensive. Ducomet (34), in 1903, recorded its appearance under the name of "brulure" in the southwestern part of France. More recently the publication of a paper by Zhavoronkov (162) would seem to imply its distribution in Russia.

In general, leaf blight is a disease characteristic of the later stages in the development of the host. During the season of 1920, when the writer had occasion to follow its development in the sweetcorn fields of the western part of Long Island, it made its appearance toward the end of August. On plants examined on August 20, elongated straw-colored spots were present especially on the lower leaves, varying from 2 to 4 mm. in width, and from 5 to 15 mm. in length. The tissues involved in these spots were altogether dry. Usually at this stage the dry areas were delimited from the surrounding healthy parts by a brownish margin, quite conspicuous and distinct in some instances and barely distinguishable or absent in others. As the season progressed the affected areas rapidly increased in size, individual lesions frequently measuring more than 4 cm. in width and 10 cm. in length by September 16. (Pl. 24, A.) By the coalescences of the enlarging spots, extensive areas embracing often considerable portions of the leaf were found to be involved. Toward the end of September the foliage was withered to such an extent that some of the plants appeared, as some writers have suggested, as if affected by a frost.

When leaf blight appears as late in the season as in 1920, which probably was very nearly typical in regard to the development of the trouble at least for the northeastern section of the United States, the resultant economic damage is not likely to be serious. In most ordinary seasons Peck's (107) observation that *Helminthosporium inconspicuum* "seems to attack on lower leaves with vitality already impaired; not very noxious as it only hastens death of leaves by a few days or weeks," perhaps is not without a good deal of justification. Nevertheless, in other seasons, apparently as a result of weather conditions favorable to the fungus, the disease may make its appearance while the plant is still relatively young, and thus cause very considerable destruction. Thus Ducomet (34) records a severe outbreak in France in 1900 during an exceptionally wet season. According to this writer the foliar lesions appear when the plants are only 0.5 to 0.6 meters high. The destructiveness of the disease in 1903 in the States of Connecticut and Delaware apparently was associated also with its early appearance, Clinton (21) finding "many fields looking in August and September as if struck by early frost; heavy attack due to unfavorable season." In New South Wales the blight is reported (118) to appear invariably in all the late plantings of maize when the heavy autumn rains set in, particularly on low-lying situations. In the season of 1915 (118), however—

it appeared very much earlier owing to the phenomenally wet spring and early summer. Many areas were completely destroyed on account of the leaves being killed off long before the plants were half grown. Where cobbing had been well advanced the effects were not so serious.

Equally severe epiphytotics apparently are not uncommon in the Philippines, where Reinking (117) found the disease sometimes extremely destructive, entire plots of field corn and sweetcorn having been ruined by it.

Several weeks after the initiation of a foliar lesion, when the affected tract usually exceeds 5 cm. in length, a grayish, greenish efflorescence makes its appearance in the center of the withered area, becoming gradually more extensive with the continued enlargement of the latter. This efflorescence consists of the numerous fructifications of the fungus, which, in spite of the statement of Cooke and Ellis (24), are more readily perceived with the naked eye than the fructifications of the majority of the species of *Helminthosporium* developing on the foliage of grasses. When such material is examined under the microscope the fructifications can be seen emerging in groups of 2 to 6, always from the stomata. The olivaceous conidiophores (Pl. 24, Ca-g) usually measure from 7.5 to 9.0  $\mu$  in width, although Saccardo (128, v. 4, p. 420-421) gives 6  $\mu$  for this dimension. His characterization of these structures as "3-pauciseptatis" is more nearly correct, as the number of septa varies usually from 2 to 4. As the sporophores have been found to attain a length of 260  $\mu$  (Pl. 24, Cf) or more, Saccardo's (128) figure, 150  $\mu$ , being apparently a decided understatement of this dimension, the intervals between the septa, compared to those of most other species, are relatively large.

The spores of the fungus, which are quite characteristic, have been described and figured in a great variety of ways, but rarely altogether correctly. As shown in Plate 24, Ba-p, drawn from material derived from diseased leaves of sweet corn collected on Long Island, they vary considerably in size and shape. The measurements for length and width, ranging from 45 to 132  $\mu$  (Pl. 24, Bl, o) and from 15 to 25  $\mu$  (pl. 24, Bi, o), respectively, agree fairly well with those given by other authors: Saccardo (128) 85 to 92 by 20 to 24  $\mu$ ; Ducomet (34) 65 to 95 by 20 to 25  $\mu$ ; Cooke and Ellis (24), and Schwarze (130) 80 to 120 by 20  $\mu$ ; Butler (19) 80 to 120 by 20 to 24  $\mu$ ; and Massee (90) 80 to 140 by 20 to 26  $\mu$ . In shape the spores are typically straight or slightly curved, widest near the middle and tapering decidedly toward the ends. The proximal portion of the spore may taper toward the hilum somewhat in the manner of a cone (Pl. 24, Bf, h, k) although a tendency toward the basal end being rounded off usually is discernible (Pl. 24, Bb, i) and not infrequently quite pronounced (Pl. 24, Bm, n). In any case, however, the shape defined by Saccardo as "perfecte fusoides utrinque acutis" is never realized, because the apical end of the spore is always rounded, even where the distal segment is very considerably inferior in width to the middle segment. Certainly, conidia like those figured by Saccardo (126, pl. 824) with the basal and apical ends drawn out into attenuated beaks, have never been observed by the writer. Nor have any been observed entirely comparable with those figured by Massee, with the acumination of the ends considerably exaggerated and the septation so close that the segments appear more than twice as wide as they are long. On the other hand, Schwarze's (130) figures represent them as rather too blunt, a fact for which the evident use of dead herbarium material may, perhaps, largely be held responsible.

The number of septa in the spores was found to vary from 1 to 8 in the material collected by the writer. Inasmuch as the 1 or 2 septate individual spores are manifestly undersized, the correspondence with the numbers given by most authors is, on the whole, satisfactory—Saccardo

(128), 5 to 8; Ducomet (34), 3 to 8; Butler (19), 3 to 7; although the range 3 to 5 given by Cooke and Ellis (24), while perhaps expressing an average condition, would not seem to be large enough. It may be noted that the septa are somewhat tardy in making their appearance and, as a result, many of the spores when newly proliferated show segments of a length not usual in species of similar dimensions. (Pl. 24, Ba, g, h.) The peripheral spore wall always is relatively thin, the accounts of Saccardo (128) and Schwarze (130), for example, as well as figures like those of Massee (90) and of Smith (133), describing or illustrating a thick membrane, being apparently based upon dead material. As usual in species of *Helminthosporium*, the thin peripheral wall is associated with a relatively light color of the conidia. This color varies from a subhyaline light fuliginous tint when the spores are newly proliferated to a moderate fuliginous, greenish yellow, yellowish brown, or pale olive when they are fully mature. The dark olivaceous color, characteristic of the conidia of *Helminthosporium sativum*, *H. monoceras*, or *H. vagans*, is never approached, descriptive phrases like that given, for example, by Saccardo (128), "olivaceofuscis," indicating such approximation, being evidently quite erroneous and misleading.

A morphological feature that the writer feels inclined to emphasize, particularly as a diagnostic character useful in separating *Helminthosporium turcicum* from most of the congeneric forms with which the student of economic botany has to deal, is the protruding hilum. (Pl. 25, C.) This apiculate basal protuberance, while rather minute, is uniformly present, regardless of whether the proximal portion of the spore is distinctly tapering or more nearly rounded. It must, therefore, be considered apart from the basal contour of the conidium. That it has not been mentioned in the writings of previous workers is somewhat surprising. Ideta (65), it is true, figured the proximal cell as being conspicuously constricted at the base. If this attenuated portion was intended to represent the part of the conidium by which the latter is inserted on the conidiophore, its proportions would seem, in view of the condition obtaining in American material, greatly exaggerated.

As has been pointed out or figured in the publications of Ducomet (34), Smith (133), Butler (19), Reinking (117), and Zhavoronkov (162), the spores of *Helminthosporium turcicum* germinate regularly by the production of two polar germ tubes, one from each end. (Pl. 15, Db, c.) Occasionally an intermediate segment may proliferate one or several lateral germ tubes. Such atypical germination apparently is more likely to occur with newly proliferated subhyaline conidia (pl. 25, Da, d), or with abnormally curved spores than with fully mature spores of typical shape; and sometimes occurs as the result of injury and death to one of the end cells.

The fungus develops well on the media usually employed in laboratories. On substrata rich in organic food materials, as for example, potato glucose agar, a luxuriant growth of grayish black aerial mycelium is produced. On substrata poorer in organic substances, like tap-water agar, growth is less luxuriant, but may be studied to better advantage. The embedded mycelium anastomoses abundantly with the resultant production of numerous complexes consisting of dark brown lobulate segments. The sporophores arise from the prostrate fuliginous hyphae that compose a large portion of the aerial growth. They are somewhat inferior in diameter to those developing in nature, measuring approximately 6  $\mu$  in width, but, at the same time, are considerably longer. As the first spore

the spores of the latter type have exhibited the irregularity in question. When such conidia germinate, the surviving segments collectively produce 1 or 2 polar germ tubes, which traverse the neighboring dead segments and their degenerate swollen envelopes like so much inert material, to emerge usually, though not always, from the ends of the spore. In conidia of which all segments are in a living condition, the juxtaposed portions of segment membranes that constitute the septa normally undergo no degeneration; at least until the germ tubes have attained extensive development.

#### HELMINTHOSPORIUM LEERSII ATKINSON

In 1897, Atkinson (4) described as *Helminthosporium leersii*, a fungus collected on leaves of *Lecrsia virginica* Willd. (= *Homolocenchrus virginicus* [Willd.] Britton) at Auburn, Ala., September 13, 1891:

Spots irregularly oblong, amphigenous, at first dark brown, then dirty white with dark brown border. Hyphae amphigenous, brown, irregularly nodulose or flexuous, 200 to 350 by 4 to 6  $\mu$ . Conidia slightly curved, 5 to 9 septate, elliptical, faintly fuliginous.

Although the form was later included in Earle's list of Alabama fungi (92), it seems to have escaped the attention of botanists in other states. Nevertheless, the parasite appears to be widely distributed, as the writer found it of frequent occurrence on the leaf blades of *L. virginica* near Meriden, Conn., in September, 1920, as well as in the vicinity of Washington, D. C., during the summer of 1921.

The first indication of attack becomes evident as a minute brown spot often not exceeding 1 mm. in length. (Pl. 26, A, C.) As this spot enlarges, the central area for some time remains dark brown, while the discoloration at the margin merges insensibly into the light green of the surrounding healthy tissue. With further increase in size, the tissues in the center succumb, their dark brown in the meantime fading to a dirty straw color. As the line of demarcation between the gray central region and the peripheral brown zone is sharply defined, a foliar lesion of the eye spot type (Pl. 26, A, C) results. The parasite seems to hasten, if not to cause, the death of the older leaves. When the latter have withered, either in whole or in part, the fructifications make their appearance, scattered sparsely, first near the center of the spot but later also beyond the margin.

According to Atkinson (4) *Helminthosporium leersii* is "near *H. turcicum* but hyphae and conidia more slender." It seems questionable with which one of a number of congeneric organisms this author intended to compare the fungus. Certainly, no striking similarity to the parasite causing leaf blight of maize is discernible with respect either to the dimensions or to the shape of spores and sporophores. In the material collected by the writer, the latter were found to arise singly or more rarely in pairs from between the epidermal cells of the host. (Pl. 26, Fa, b.) The moderately fuliginous conidia (Pl. 26, Ba-1), measuring from 11 to 14  $\mu$  in diameter by 50 to 95  $\mu$  in length, were found to contain from 3 to 12 cross walls, never associated with perceptible constrictions in the unusually thin peripheral wall. Irregularities in the insertion of cross walls, resulting in muriformly septate conditions are present as in the spores of *H. sativum*. (Pl. 26, Bk.) As in *H. sativum* also, germination normally takes place by the production of two polar germ tubes. (Pl. 26, Da-c.) However, owing to the fragility of the

external wall, the intermediate segments frequently are exposed and sometimes entirely liberated in the course of manipulation, with the result that abnormal germination is much more common than in most congeneric forms. The germ tubes usually attain a considerable length before branching, thus differing in this respect from those of *H. cynodontis*, which frequently proliferate a branch at the very point of origin. The hilum in the spores of *H. leersii* is represented by a scar generally contained within the contour of the basal end, although occasionally it may be seen to protrude slightly. In any case, a basal modification like that characteristic of the conidia of *H. monoceras* is never present; nor is the hilum a distinctly protruding structure as, for example, in *H. turcicum*.

When grown on artificial media the fungus, unlike the other species similar to *Helminthosporium sativum*, develops a dense gray aerial mycelium, the rate of enlargement being relatively slow. This slow development is associated with a peculiarity in manner of growth at the margins that is more or less characteristic and has not been observed in other species. The imbedded hyphae, although ramifying profusely, remain short, thus giving rise to an intricate system of short, rather swollen elements of which Plate 26, E, represents merely an incipient stage. Here and there a relatively delicate hypha (Pl. 26, Ea-b) grows out into the air and by curving downward brings its tip in contact with the substratum. From the tip numerous short branches are soon proliferated which, by continued ramification, again yield an intricate system of hyphae.

#### HELMINTHOSPORIUM CYNODONTIS MARIGNONI

In 1909, Marignoni (89) described as *Helminthosporium cynodontis* a fungus occurring on dry leaves of *Cynodon dactylon* L. (= *Capriola dactylon* [L.] Kuntze) near Schio in northern Italy. In the brief diagnosis given by Saccardo (128, v. 22, p. 1394), the species is thus characterized:

Effusum, atro-olivaceum v. fuliginereum; conidiophoris aggregatis, laxis, simplicibus, parce septatis, tortuosis,  $80-150 \times 6-7$ ; conidiis elongatis, utrinque rotundatis plerumque octo-septatis, fuliginis,  $60-75 \times 12-14$ .

Apparently the fungus has not been reported again, as the subsequent literature appears to contain no reference either to Marignoni's binomial, or to any species of *Helminthosporium* occurring on Bermuda grass and answering the description given above. The writer nevertheless is convinced that the parasite is exceedingly common throughout the southeastern section of the United States where the host is everywhere present in the fields and on the roadsides as a noxious weed. In Florida, near Wauchula, Fort Myers, Tampa, and Gainesville, during the months of February, March, and April, 1921, it was found difficult to collect specimens of *Cynodon dactylon*, not bearing fructifications of the fungus in considerable abundance. (Pl. 27, A.)

Although the writer has not been able to consult Marignoni's (89) illustrated publication, the American form answers sufficiently well in morphological detail to Saccardo's (128) account of *Helminthosporium cynodontis* that, in view of its abundant occurrence on the same host, it can at least provisionally be regarded as belonging to this species. In the Florida material, the conidiophores (Pl. 27, Da-g) which are dark brown in color, emerge singly or in pairs from stomata or between



epidermal cells, and thus show less tendency to occur in groups than those of most congeneric species. They measure from 50 to 150  $\mu$  in length, and from 4 to 6  $\mu$ , typically 5  $\mu$ , in diameter, the range in length agreeing well with that given in Saccardo's diagnosis. On the other hand, the measure of agreement in diameter of sporophore is somewhat less satisfactory than might be expected in a dimension exhibiting relatively little variability. The number of septa in the conidiophores range from 2 to 5, depending largely on the length of these structures.

The conidia (Pl. 27, Ba-u) of the American form, measuring 11 to 14 by 27 to 80  $\mu$ , are straight or more frequently somewhat curved; widest near the middle from which they taper slightly toward the abruptly rounded ends; subhyaline to fuliginous in color, never brown or dark olivaceous; and 3 to 9 septate, the septa not being associated with constrictions in the relatively thin peripheral wall. On being mounted in water they germinate promptly by the production of two polar germ tubes approximately 3  $\mu$  in thickness, one from the apex and the other in immediate proximity to the hilum that can be distinguished within the rounded contour of the basal cell. (Pl. 27, Ca-i.) Very frequently a lateral branch is produced near the origin of the germ tube, thus often simulating the appearance of two germ tubes. (Pl. 27, Cc, e.) The spores are obviously of the same type as those of *Helminthosporium sativum*, from which they differ markedly however, in size, thickness of peripheral wall, and depth of coloration. They are inferior also to those of *H. leersii* in length and number of septa; and to those of *H. micropus* in width, besides lacking altogether the peculiar modification of the basal segment characteristic of the latter species. The fungus grows on artificial media, producing a moderate quantity of light gray, fluffy mycelium, especially at some distance from the point at which the inoculum was planted. It has not been observed to sporulate in culture on media ordinarily employed in laboratories, although the production of spores could probably be induced by providing more suitable substrata.

It may be mentioned that while the fructifications of *Helminthosporium cynodontis* are found occurring most abundantly on moribund or withered leaves of *Cynodon dactylon*, the fungus also has been collected repeatedly on *Eleusine indica* in Florida in the spring of 1921. In the vicinity of Washington, where the parasite was present in moderate quantity on Bermuda grass from August to October, 1921, it was not found on goose grass, indicating that the latter host is somewhat less favorable for its development. A form morphologically very similar and probably identical has been found to occur very consistently on withered leaves of *Muhlenbergia mexicana* (L.) Trin. collected in the vicinity of New York City and Washington, D. C. Although further investigation of the host range is necessary, indications are not wanting that the species will eventually be found on more than a few members of the Gramineae.

#### HELMINTHOSPORIUM MICROPUS, N. SP.

About the middle of April, 1921, the writer observed a peculiar disease affecting young plants belonging to a species of *Paspalum*, provisionally identified as *Paspalum boschanum* Flügge, that was found common in moist, poorly cultivated fields near Wauchula, Fla. The trouble occurred on seedlings from 3 to 6 inches in height, first becoming apparent on the tender young foliage as a localized wilting. (Pl. 28, A.) Portions of foliar tissue from a few millimeters to several centimeters in length, and

from 2 to 4 mm. in width, were found in an entirely collapsed condition. (Pl. 28, B.) The absence of any indication of discoloration and the entire loss of mechanical stiffness combined to present an appearance such as might be brought about, for example, by scalding with boiling water. Soon after the wilting became visible, the portions of leaf involved dried out completely, becoming somewhat shrunken, dark in color and crisp in texture. Usually the death of the entire leaf blade ensued within a week, as much apparently because of the interruption of the vascular elements by the enlargement and multiplication of infected regions as because of the extension of the trouble to healthy parts. On the older leaves the disease was found less destructive, the injury tending to be restricted to more definitely circumscribed elliptical spots, varying from 2 to 10 mm. in length, and not infrequently delimited by a brownish margin. Nevertheless, these older leaves likewise slowly succumbed, the withering beginning at the tip and gradually progressing toward the base.

On examining the dead foliage under the microscope, it was found that the regions involved in the lesions bore numerous fructifications of a well defined species of *Helminthosporium*. The first conidiophores to appear after the death of the tissue seemed usually to emerge from the stomata (Pl. 28, Ea-g), although later they could be found emerging between epidermal cells as well. Except that the sporophores are smaller in diameter than might be expected from the size of the spores, they exhibit no distinctive characteristic.

The conidia (Pl. 28, Ca-m) on the other hand are decidedly characteristic and can be readily distinguished from those of any congeneric species which the writer has examined. Generally subhyaline or slightly fuliginous, they resemble in respect to coloration the spores of *Helminthosporium teres*, *H. bromi*, *H. giganteum*, and *H. tritici-repentis*. Unlike the conidia of any of these fungi, however, they are typically more or less curved, a fact which together with the mode of germination by the production of two polar germ tubes (Pl. 28, Da-c) suggests comparison with *H. leersii*, *H. cynodontis*, and *H. turcicum*. From the spores of the fungus causing leaf blight of maize, those of the parasite on *Paspalum boscianum* are readily distinguished by their smaller dimensions, a very perceptible difference obtaining in respect to length and diameter. As the number of septa in the conidia are approximately equal, or even somewhat greater in the fungus thriving on *P. boscianum*, a very pronounced difference in massiveness between the spore segments of the two species is readily apparent. In general shape, the conidia of the parasite on *Paspalum boscianum* are less inclined to taper toward the ends than those of the *H. turcicum*, frequently all the segments except the terminal ones being of nearly the same diameter. The most characteristic feature, however, is found in the shape of the proximal end of the spore, which tapers quite abruptly and uniformly from the basal septum into a nearly cylindrical short prolongation, terminating abruptly in the flat hilum. Because of this curious modification, the contour of the peripheral wall usually exhibits a slight, barely perceptible, reentrant curve.

Among the species of *Helminthosporium* discussed in this paper the form on *Paspalum boscianum* is altogether unique in causing under natural conditions a violent wilting effect on the tissues of its host. The only instance known to the writer of similar pathological symptoms attributable to a congeneric species is the wilting produced experi-

diameter of its spores. Nor does it appear possible on morphological grounds to identify it with other species of *Helminthosporium*, including the numerous saprophytic types that have been referred to the genus. The specific name *rostratum* is suggested as descriptive of a conspicuous feature characteristic of many of the conidia.

#### DIAGNOSIS

#### *Helminthosporium rostratum*, n. sp.

Occurring on the dry leaves of *Eragrostis major*, Host.

Conidiophores dark olivaceous, emerging singly or in groups of 2 to 5 from stomata or between epidermal cells, the swollen bases often more or less united; measuring 6 to 8 by 40 to 180  $\mu$ ; 1 to 6 septate, the septa separated by intervals of 15 to 40  $\mu$ ; proliferating the first spore 40 to 140  $\mu$  from the base, and successive spores at intervals of 10 to 30  $\mu$ , at the apices of well-defined geniculations.

Conidia, when mature, dark olivaceous; straight or less frequently somewhat curved; often short, widest at or somewhat below the middle, tapering moderately or more markedly toward both ends, the hemispherical apex abruptly rounded off, the basal end somewhat more acute, often exhibiting a rounded conical contour; or less frequently produced at the tip into a more or less elongated rostrate prolongation. The elliptical spores 3 to 9 septate, the rostrate types usually 8 to 15 septate, the proximal cross wall occasionally associated with a perceptible constriction in the peripheral wall. The basal septum often, and the distal septum less frequently, appearing darker and thicker than the intermediate cross walls, such modification not unusually associated with a more dilute coloration of the delimited basal or distal segments. Peripheral wall thick except in two small subhyaline regions, one at apex, the other surrounding the conspicuously protruding hilum at the base. Mature spores germinating by the production of two polar germ tubes, one from each of the subhyaline thin-walled regions; immature spores often producing germ tubes also from intermediate segments. Measuring 14 to 22  $\mu$  in diameter by 32 to 184  $\mu$  in length.

HABITAT.—Collected near Washington, D. C., September and October, 1921.

#### HELMINTHOSPORIUM ORYZAE B. DE H.

*Helminthosporium macrocarpum* of von Thümen not Greville.

*Helminthosporium oryzae* Miyabe & Hori 1901, in *Nôji Shikenjo Hôkoku*, no. 18, p. 67-81.

Probably the earliest record of the occurrence on rice of a species of *Helminthosporium* resembling the form now recognized as a widely distributed parasite on this cereal may be credited to von Thümen (150). This writer in a paper published in 1889 reported *Helminthosporium macrocarpum* Grev. as not infrequently appearing indiscriminately on dead parts of rice plants immediately after the tissues involved have ceased to live. To the presence of the fungus was attributed a discolored appearance of the crop that had occasioned popular discussion of "attack" and "sooty mould." Von Thümen believed that the fungus nevertheless is not the cause of any disease, but that it makes its appearance rather as the result of disease or as a saprophyte accompanying entirely normal maturation.

While the various saprophytic organisms that have been referred to *Helminthosporium macrocarpum* by different authors in all probability are not specifically identical, it may be assumed that such reference implies a moderately close correspondence to the diagnosis of Greville's species. In most details, indeed, this diagnosis is not widely at variance with descriptions of the fungus causing leaf spot of rice. A significant departure is evident, however, in the width of the sporophore, which in *H. macrocarpum* measures 15 to 20  $\mu$ , thus equaling or slightly exceeding the diameter of the conidia. The identity of the fungus observed by von Thümen thus is rendered somewhat doubtful. In any event it seems clear that no useful purpose could be served by associating Greville's binomial with the parasite on rice.

In 1900 van Breda de Haan (16) described from Java as *Helminthosporium oryzae*, a fungus producing spots on living rice leaves, the affected areas being entirely dry in the center and surrounded by a brown margin. The brown conidiophores arising from the under side of the leaves, according to his characterization, bear large, fuliginous fusiform acrogenous 6- to 9-celled conidia, measuring 16 by 90  $\mu$  and germinating from both end cells. The fungus which had also been found on the fruits of rice, the author, evidently influenced by von Thümen's paper, regarded as probably identical with *H. macrocarpum* Grev.

The next year (1901) Hori (62) gave an account of the same disease in Japan, and apparently without knowing of van Breda de Haan's paper, named the parasite *Helminthosporium oryzae* Miyabe and Hori. The fungus has since been reported from Japan by Yoshino (161), Kurosawa (80), and others, while in more recent years Suematsu (146) has investigated its cultural characters, and Nishikado and Miyake (94) have studied methods for its control. An illustrated account of the disease and the parasite is given in Ideta's large handbook (65). An unidentified species of *Helminthosporium* on rice was reported from the Straits and Federated Malay States by Gallagher (46), from Madras by Sundararaman (147), from Uganda by Snowden (135), from Ceylon by Bryce (17), from Cochin-China by Vincens (155), as well as from the Philippines by Reinking (117). In a recent note, Ocfemia (96) states that in 1918 he observed a seedling and leaf blight attributable to *H. oryzae* doing considerable damage to rice in the Philippines. Farneti (39) ascribed the "brusone" disease of rice in Italy to a fungus highly variable in its morphological characteristics and pathological manifestations; presumably appearing as either *Piricularia grisea*, *P. oryzae*, *H. turcicum*, or *H. oryzae*, its form in any particular case being contingent on the host plant, the organ attacked, and the environment.

Beyond a statement in Ocfemia's (96) note that the "sesame spot disease" of rice caused by *Helminthosporium oryzae* was observed by W. H. Tisdale in Louisiana in 1920, the American literature does not seem to contain any reference to the occurrence of the parasite in this country. Dr. Tisdale has advised the writer that the *Helminthosporium* leaf spot is of not uncommon occurrence in the rice fields of Louisiana and Texas, and has kindly supplied specimens of affected mature rice inflorescences collected in this locality on September 15, 1920. An examination of these specimens showed that the fungus occurs on the glumes at first as a grayish efflorescence, and later, because of continued development, as a black velvety mat, somewhat similar in texture to the crustose growth of *H. ravenelii* on *Sporobolus indica* but much less extensive. (Pl. 30, A.) Through the courtesy of Mr. Ocfemia, pure cultures of the fungus originally isolated from Louisiana material were obtained, as well as specimens of rice leaves from experimental plants artificially inoculated. The leaves bore an abundance of dark brown or reddish brown spots, longitudinally elongated, the larger ones measuring up to 0.5 by 3.0 mm., and showing a small straw-colored area in the center. (Pl. 30, B.) No indication of an etiolated zone surrounding the foliar spot like that characteristic, for example, of the blotch caused by *H. bromi*, was present, the discoloration caused by the fungus manifestly resembling that produced by *H. leersii* on *Leersia virginica*, and belonging to the type that in other instances has suggested the term "eye spot." In pathological symptoms the American parasite thus resembles the fungi described from Java (16) and from Japan (62).

The correspondence in morphological features between the three forms is sufficiently close to warrant regarding them, at least provisionally, as specifically identical. To be sure, in respect to the dimensions of conidiophore and conidium as well as to numerical range in spore septation, Breda de Haan's (16) account is not altogether in perfect agreement with Hori's description; and either account reveals shortcomings when considered in relation to the American fungus. However, if the more extreme and relatively infrequent expressions of length and septation are disregarded, the differences are not especially large. Perhaps the most serious discrepancy is found in diameter of conidium, the measurements given in Hori's account, 16 to 22  $\mu$ , exceeding the measurement given by Breda de Haan and also the measurements obtained from American material, by somewhat more than can readily be referred to ordinary variability, in view of the comparative constancy generally characteristic of this dimension within a particular species. Although Ideta's (65) figures indicate that the Japanese fungus is at least of the same general type as the American form, a brief morphological account based on material from Louisiana nevertheless may not be superfluous.

The black, velvety, mycelial mats on the glumes of affected spikelets, which are found distributed irregularly and usually rather sparsely through otherwise healthy panicles (Pl. 30, A), are composed of prostrate hyphae and more or less erect sporophores. The former, which communicate directly with the mycelium in the tissues of the host, when well developed, show abundant branching and anastomosis and are composed of short segments, dark brown or olivaceous in color, more or less inflated or lobulate, and measuring from 8 to 15  $\mu$  or more in diameter (Pl. 30, D). The sporophores arise as lateral branches from these hyphae, which indeed they resemble toward the base, in possessing a dark olivaceous color, and in showing a tendency toward ramification. (Pl. 30, D.) Some distance from their base, the sporophores gradually change from an olivaceous color to a light fuliginous hue, and at the tip may even be subhyaline. They vary in width from 4 to 8  $\mu$ , and in length from 150 to 600  $\mu$  or more, depending on the age of the growth. The scar marking the point of attachment of the first spore is found above the olivaceous proximal portion of the sporophore, usually not less than 200  $\mu$  from the base; successive scars occur at relatively long intervals (10 to 90  $\mu$ ) at geniculations not always well defined or conspicuous.

As collected on rice plants naturally infected, the conidia measure 11 to 17  $\mu$  in diameter by 35 to 170  $\mu$  in length. The larger ones like those shown in Plate 30, Ca, b, and containing as many as 13 septa, appear to be produced for the most part on well developed mats of sporophores occurring on the glumes; while the less extreme sizes (Pl. 30, Cc, m) are associated with the scattered fructifications on the glumes or leaves. Apparently because of the absence of the longer spores from diseased leaves, those of more moderate length have been regarded as characteristic of the parasite, the one figured in Plate 30, Cf, for example, fitting almost exactly the description given by van Breda de Haan. Typically the spores are slightly curved, widest at the middle or somewhat below the middle; the distal portion tapering toward the hemispherical apex where its width approximates half the median width; the proximal portion tapering toward the base, which is similarly rounded off, although the diminution in diameter is usually perceptibly less. When fully mature they are fuliginous or brownish and provided with a moderately thin peripheral wall that is further attenuated at the apex as well as imme-

diately around the rather inconspicuous hilum visible within the contour of the base. Normal germination of mature spores proceeds by the proliferation of two polar germ tubes, one from each of the thin-walled regions (Pl. 30, Cd, e); while less mature, subhyaline spores may produce germ tubes from intermediate segments as well (Pl. 30, Ci, k).

On tap-water agar the fungus grows sparsely, producing conidiophores and conidia (Pl. 31, Aa, b; B) which, while somewhat narrower and more nearly colorless, are essentially similar to those found in nature. However, on other substrata, as, for example, potato glucose agar, growth is very profuse, and owing to the blackish olivaceous color and velvety or felt-like texture of the mycelium, somewhat similar in general appearance to the growth of *Helminthosporium sativum* (Pl. 31, D). The spores, although of a shape and size not entirely unlike those found in nature (Pl. 31, Cd, Da) in spite of many markedly irregular examples (Pl. 31, Db, c) frequently exhibit such pronounced departures in respect to coloration as to suggest the suspicion that one might be dealing with a separate species. Instead of a uniform fuliginous, brownish or dark olivaceous color, they show in the same culture all gradations from subhyaline to deep olivaceous, and in some instances are so nearly black as to appear opaque even when a moderately bright illumination is used. Associated with the dark coloration is a peripheral wall conspicuously and uniformly thick except over the apical and basal regions involved in germination. (Pl. 31, Ca.) Frequently one or both end segments are altogether subhyaline (Pl. 31, Cb, c, e, Db), in sharp contrast to the other segments, and occasionally one or more intermediate segments show similar differentiation (Pl. 30, Cb, e). But whatever its position, the subhyaline segment is always set off from the dark segments by greatly accentuated heavy septa. Manifestly, coloration, and, in a smaller measure, structure are contingent here upon conditions not usually present when the fungus grows on rice plants under natural conditions or on tap-water agar in artificial culture.

In general morphological characteristics *Helminthosporium oryzae* suggests comparison with *H. sativum*, *H. monoceras*, and *H. turcicum*. From *H. sativum* it may be distinguished by the greater length and the more pronounced tapering toward apex and base, characteristic of its conidia. On the other hand, the conidia of *H. oryzae* taper less markedly toward the basal end than the homologous structure of the parasites on barnyard grass and on corn; and the hilum is contained within the contour of the base, not protruding as in the latter two species. Certainly Farneti's (39) belief that *H. oryzae* and *H. turcicum* represent specifically identical fungi seems altogether incredible, in view of the well-defined morphological differences between the parasite affecting rice and the form causing leaf-blight of maize.

During recent years methods for controlling the brown-spot disease have been investigated by Nishikado and Miyake (94) in Japan, where it constitutes one of the most serious troubles affecting rice culture. In the province of Okayama, for example, 90 per cent of the seedlings in the seed-bed were found affected to a greater or less extent, and sometimes practically all the seedlings bore lesions, making it difficult to find entirely healthy specimens. Controlled experiments brought to light the fact that this very general seedling infection was largely attributable to infected or contaminated seed. It was found that the spores of *Helminthosporium oryzae* are killed by immersion in water at a temperature of 51° C. for 10 minutes, while air-dry rice seed is not injured by immersion for 10 to 15

minutes in water at a temperature of 54 to 55° C. As a practical method of control, treatment of rice seed in water for 10 minutes at 52° C., or for 5 minutes at 54° after preliminary soaking for 24 hours at room temperature, was recommended.

The disinfection of rice seed by other methods, including possibly the hot air method devised by Atanasoff and Johnson (3) and treatment with various organic mercury compounds, presents a profitable field for further research. In this connection it may be mentioned, however, that the conidia of *Helminthosporium oryzae* appear to remain viable relatively long periods, the writer having germinated, in September, 1921, spores from material collected in Louisiana on October 1, 1920, nearly 12 months earlier. (Pl. 30, Cd, i, k.) As a result the fungus undoubtedly is able to survive from season to season on stubble, straw, and other refuse. Although the prevention of primary seedling infection may reduce to some extent the number of secondary infections at later stages in the development of the plant, it can scarcely be expected to eliminate them altogether. As in the somewhat analogous disease of barley, wheat, and rye caused by *H. sativum*, generally approved agricultural practices making for soil sanitation should prove of some value.

In nature the fungus does not seem to have been found to attack plants other than rice, although on artificial inoculation Ocfemia (97) secured infection of 31 species of grasses belonging to 23 genera. Evidently this represents another instance in which the experimental host range is more an expression of the rigorous conditions attending the procedure followed than of significant parasitic relationships.

Since the submission of this paper for publication, a valuable account of the *Helminthosporium* disease of rice has been published in English by Nisikado and Miyake (94a), not only incorporating the results of their own comprehensive studies but also including suggestive allusions to a considerable volume of investigations, the reports of which have not hitherto become generally known among readers of the European languages. Their full account of the morphology, pathogenicity, and cultural characters of the fungus, and their abundant illustrations, leave no doubt that the parasite found destructive in Japan and presumably in many other rice-growing countries of the Orient is altogether identical with the one discussed in the foregoing paragraphs. The lack of close agreement in measurements of conidia and conidiophores given by different authors would seem to be due in large measure to the variability of the fungus under different conditions of growth both in nature and more especially in artificial culture—its behavior in this respect being again analogous to that of *Helminthosporium sativum*. In addition, the inclusion in the range of dimensions of the more extreme measurements by some authors, and their exclusion by others, have not made for any close correspondence. As a special instance, the range in diameter of the sporophore may be cited, some writers having included measurements of the inflated basal segments, or of the distended segments of the prostrate elements from which the sporophores arise, while others have excluded them. The latter course was followed in the present account, as it was believed that in a comparative treatment involving a number of species the figures thus obtained would have the greater significance.

## HELMINTHOSPORIUM CYCLOPS, N. SP.

In July, 1921, the writer collected, near Lisbon Falls, Me., specimens of *Danthonia spicata* (L.) Beauv. that appeared to be slightly affected with a leaf spot of the type caused by *Helminthosporium sativum*. Although the grass had completely headed and most of the lower basal leaves were dead, the remaining foliage was still green and in an actively vegetative condition. The dark brown or black foliar lesions, usually not exceeding 0.5 by 2 mm. in length, were found distributed very sparsely over the living foliage. On the dead basal leaves corresponding spots were observed, although considerably faded; and in a number of instances microscopic examination revealed these as the foci of fructifications of a species of *Helminthosporium* differing markedly from a small-spored congeneric form that also was present in moderate quantity. Although the fructifications of the former species were not confined to the conspicuously discolored areas, their distribution on the dead leaves was such as suggest a causal relation between the fungus and the brown foliar lesions. Owing to the small size of the leaves of *Danthonia spicata* and the tendency of the foliage to begin withering early in the course of the growing season, the matter of referring such type of foliar discoloration to a fungus not appearing on the surface until after the death of the tissues involved, and then not in great abundance, is attended with some uncertainty, as has been noted in another connection. The possible damage resulting to the host from the leaf spot in any case would appear to be quite insignificant.

The conidiophores (Pl. 32, Ea-c) of the fungus are not especially characteristic, and show little to distinguish them from those of *Helminthosporium sativum* or *H. vagans*, in size, color, or general appearance. The conidia (Pl. 32, ca-m) also resemble the analogous structures of these two species in possessing, when mature, a conspicuously thick peripheral wall, and, associated with this thick wall, a dark olivaceous color. In length they are approximately equal to the conidia of *H. sativum* and not greatly inferior to those of *H. vagans*; while in diameter they fall slightly below the spores of the former species and more considerably below those of the latter. Uniformly straight, and either nearly cylindrical or tapering more perceptibly toward the ends, they perhaps resemble most closely the spores of *H. vagans* in general shape. A specific difference readily distinguishing the spores of the form on *Danthonia spicata* from those of *H. sativum* and *H. vagans* is found in the more pronounced attenuation of the peripheral wall at the base and apex. The basal end, moreover, is distinguished by a conspicuous hilum, the largest observed in any species reported in this paper, which, because of a somewhat fanciful resemblance to an eye, has suggested the specific name *cyclops*.

The conidia (Pl. 32, Da, b) germinate readily in water, producing two polar germ tubes, which emerge, as might be anticipated, from the hyaline, thin-walled regions at the tip and immediately surrounding the hilum. In mode of germination the fungus thus again resembles *Helminthosporium sativum*. That the relationship with the latter species nevertheless is not a very close one is indicated in agar cultures by the development from the imbedded mycelium of an abundance of inflated elements altogether analogous to those observed, for example, in cultures of *H. tritici-repentis*, *H. bromi*, *H. teres*, and *H. turcicum*. On potato-dextrose agar a moderately profuse gray aerial mycelium is



produced, interspersed with a considerable number of subspherical, dark, superficial sclerotia, not unlike those produced under similar conditions by *H. bromi* and probably representing also in this instance, immature perithecia. Its analogy is not entirely misleading, it would seem that search for an ascigerous condition of this fungus might perhaps not be without success.

When cultivated on substrata containing little organic food material, as, for example, Beijerinck's agar, the fungus usually develops a variable number of sclerotia below the surface of the medium resembling the superficial ones described above, but occasionally departing from the subspherical type by growing into elongated, rather irregular bodies. (Pl. 32, A.) Microscopically, the aerial growth is relatively scant, the loose mycelium being limited to a small quantity near the point of inoculation. The larger portion of the surface is peppered with discrete fructifications such as are represented on Plate 33, Aa, Bb, C, D. Although generally noticeably smaller, the conidia developed in culture resemble in essential details those found on the host in nature. The discrete conidial fructification is, in general, of the type exemplified in *H. sativum*, but a few marked differences frequently occur. After a spore has been proliferated, it frequently grows out into an apical prolongation (Pl. 33, Ba, Bb, Da) having the same diameter as the sporophore and proliferating spores in the same way (Pl. 33, Baa, Bba, Daa-ab). Or, without any special modification it (Pl. 33, Dc) may produce a secondary spore by apical proliferation (Pl. 33, Dca). The sporophore occasionally also, shows an aberrant tendency by continuing apical growth not in the usual manner, but by a process of budding exactly similar to that occurring in the production of a conidium. (Pl. 33, Ab-Abc.) In such cases the distal part of the sporophore is similarly marked at the base by a conspicuous hilum. Obviously the fungus shares in some measure the *Alternaria*-like habit and tendency toward the obliteration of spore and sporophore manifested in *H. catenarium*.

A compound type of fructification also is produced by the fungus. In tube cultures of Beijerinck's agar, these occur in the form of erect, stiff, thread-like black structures, measuring from 0.3 to 0.5 mm. in diameter and arising usually from a slightly expanded base in the center of the loose aerial mycelium. (Pl. 32, A B.) The lower portion for a distance of about 1 mm. usually is sterile. (Pl. 32, Bd.) Above this sterile portion the fructification bristles with a dense array of sporophores resembling the discrete conidiophores and bearing similar spores. (Pl. 32, Bc.) The axial column is hard in texture and when broken and examined microscopically appears to be composed of dense white pseudo-parenchyma of which the surface layer is largely quite black but interspersed with numerous small lighter areas. Except at the abruptly truncated tip (Pl. 32, Ba) where further growth of the fructification occurs and where new sporophores (Pl. 32, Bb) are constantly being proliferated, it is impossible to recognize the hyphal origin of the axial-column. These fructifications, which appear moreover to show some similarity to the sclerotia produced by the fungus, have been seen to attain a length exceeding 1 cm. and undoubtedly could be grown much larger.

## DIAGNOSIS

***Helminthosporium cyclops*, n. sp.**

Occurring on the leaves of *Danthonia spicata* (L.) Beauv. on which it causes small dark-brown spots not usually in abundance.

Conidiophores, olivaceous, usually 3 to 7 septate, the septa occurring at intervals of 18 to 50  $\mu$ ; measuring 7 to 8  $\mu$  in diameter by 100 to 250  $\mu$  in length; producing the first spore from 80 to 160  $\mu$  from the base, and successive spores at the apices of moderately pronounced geniculations.

Conidia, dark olivaceous except in restricted subhyaline regions at apex and base, straight or rarely slightly curved, cylindrical or tapering gently toward the bluntly rounded ends, the shorter ones often ellipsoid; measuring usually 12 to 17  $\mu$  in diameter by 45 to 110  $\mu$  in length; 4 to 12 septate, the septa not usually associated with constrictions in the peripheral wall; the latter thick except in the subhyaline regions from which the two polar germ tubes are produced, one at the apex, and the other immediately surrounding the unusually large and conspicuous hilum included within the basal contour.

On agar media containing abundant organic food material, producing numbers of superficial subspherical sclerotia resembling immature perithecia of congeneric forms. On agar media containing little organic food material, developing imbedded sclerotia, discrete fructifications and compound fructifications. Conidiophores of discrete fructifications arising from more delicate subhyaline vegetative hyphae often becoming branched as a result of individual spores being produced into sporophoric prolongations and occasionally continuing growth by budding in a manner analogous to the proliferation of spores. Conidia like those produced in nature but usually shorter, ellipsoidal, rarely exceeding 18  $\mu$  in diameter and 60  $\mu$  in length.

Compound fructifications consisting of a threadlike axis 0.3 to 0.5 mm. in diameter, 10 mm. or more in length, composed of hard, white pseudoparenchyma with black mottled surface, and bearing numerous radially oriented sporophores above the somewhat expanded sterile basal portion.

**HABITAT.**—Collected near Norwood, Mass., November 7, 1920; and Lisbon Falls, Me., July 20, 1921.

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## PLATE 1<sup>1</sup>

### *Helminthosporium gramineum*

A.—Upper portion of barley plant collected near Fort Atkinson, Wis., June 25, 1921, illustrating condition soon after death. *Aa* represents abortive inflorescence with distorted awns slightly protruding laterally from the uppermost leaf sheath. The leaves show longitudinal splitting into strips as well as lack of mechanical rigidity evidenced by their drooping (*Ab*) and contorted (*Ac, d*) positions. *Ae* represents basal shreds of leaf, the remainder of which has broken off as result of brittle texture due to disease.  $\times 3/4$ .

Ba-h.—Normal spores showing variation in size, shape, and septation.  $\times 500$ .

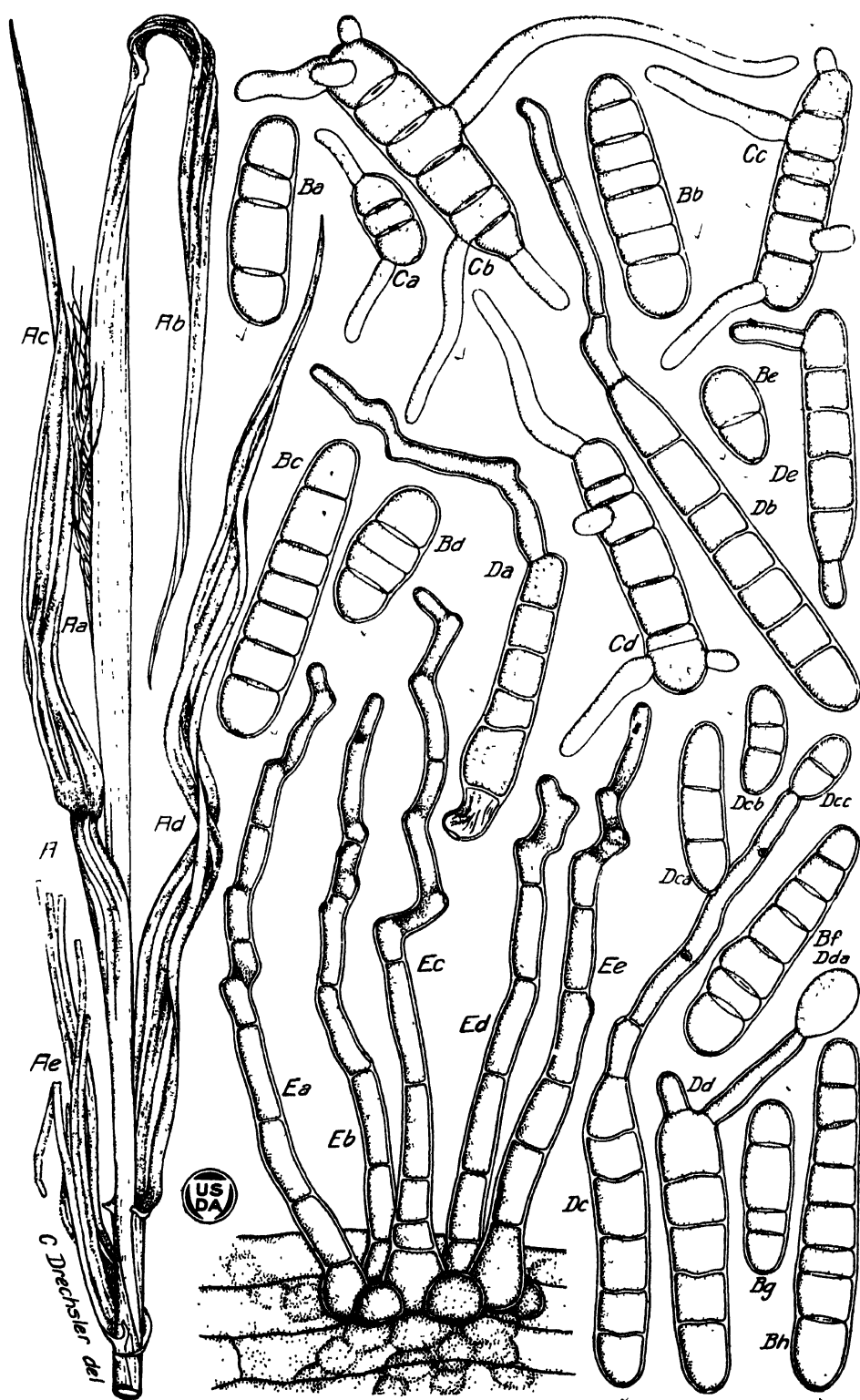
Ca-d.—Normal spores germinating in water mount, showing single germ tube produced from distal and middle segments, and 1 to 3 germ tubes produced from basal segment.  $\times 500$ .

Da-c.—Conidia from the apical segment of which has been produced a single conidiophore with scars showing places of attachment of secondary conidia; also secondary conidia *Dca-cd*. *Dd*.—Spore with two apical secondary sporophores, one showing secondary spore attached. *De*.—Spore with two secondary sporophores, one produced from basal and one from apical segments.  $\times 500$ .

Ea-e.—Group of typical sporophores emerging between distorted epidermal cells, showing enlargement of basal segment.  $\times 500$ .

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<sup>1</sup> All figures of conidia, germinating conidia, mycelia, conidiophores, asci, and ascospores in the plates accompanying this paper were drawn with the aid of a camera lucida to the same scale, and in reproduction, reduced in equal proportion to give a uniform magnification of 500 diameters. Host epidermis figured in connection with conidiophores was drawn in surface aspect with the aid of the camera lucida, and the resulting figures redrawn as if projected at an angle of 30 degrees, yielding in reproduction a magnification of 500 diameters in a longitudinal direction and approximately 250 diameters in transverse direction. Figures of pathological habit, of perithecia, of compound fructifications, and of test tube cultures were drawn at convenient magnifications, reduced in reproduction to the scales specifically indicated in the legends



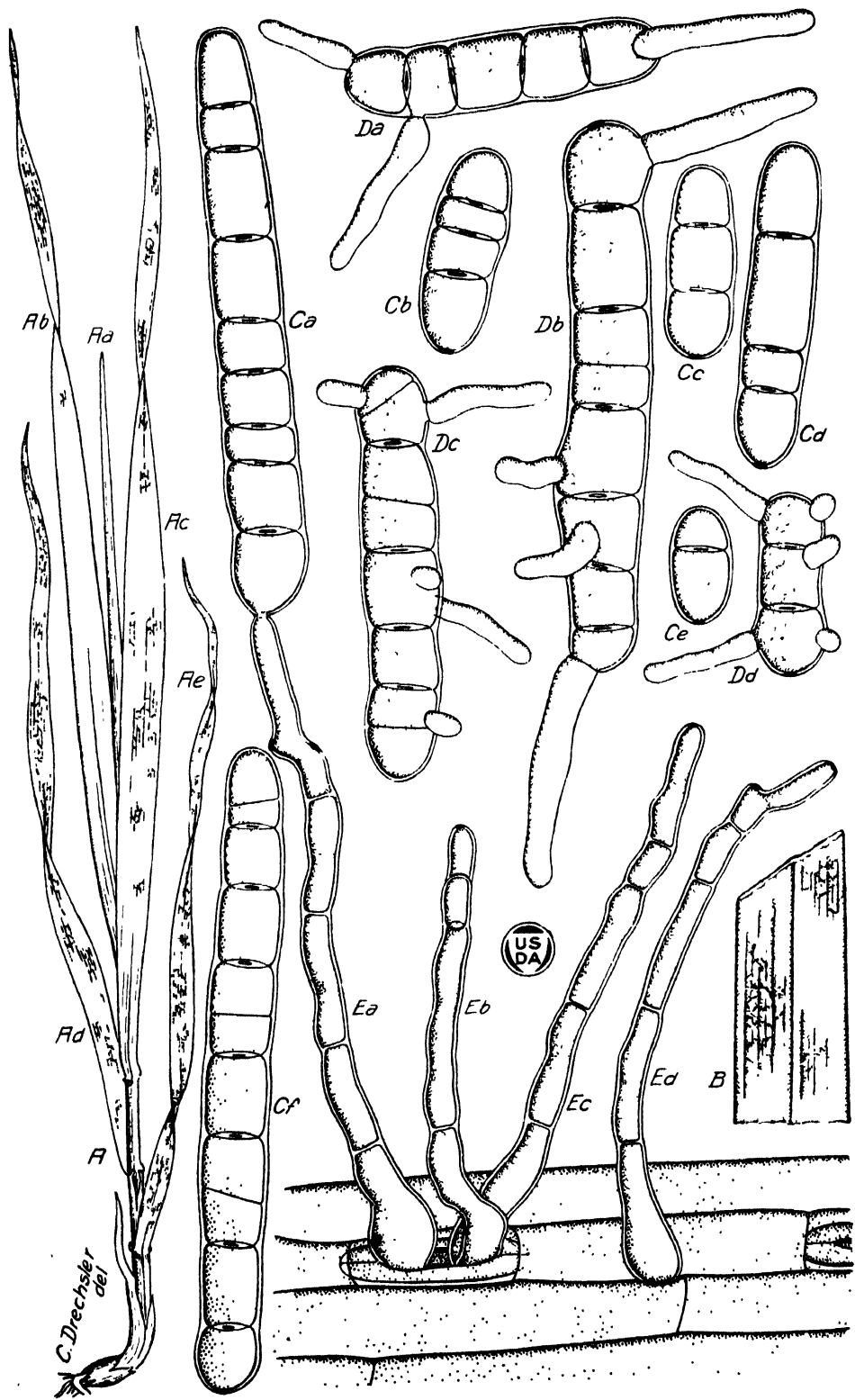


PLATE 2

*Helminthosporium levis*

A - Young volunteer barley plant showing heavy infection of net blotch as found occurring near Madison, Wis., during the earlier part of September, 1919. Youngest leaf (1a) healthy, second and third leaves (1b, 1c) showing increasing amount of infection, fourth leaf (1d) beginning to wither at the tip, fifth leaf (1e) entirely withered.  $\times 3\frac{1}{2}$

B - Portion of diseased leaf showing discoloration in characteristic irregular reticulate pattern.  $\times 5$

Ca-f - Conidia from diseased barley leaf showing variation in size, shape, and septation.  $\times 500$

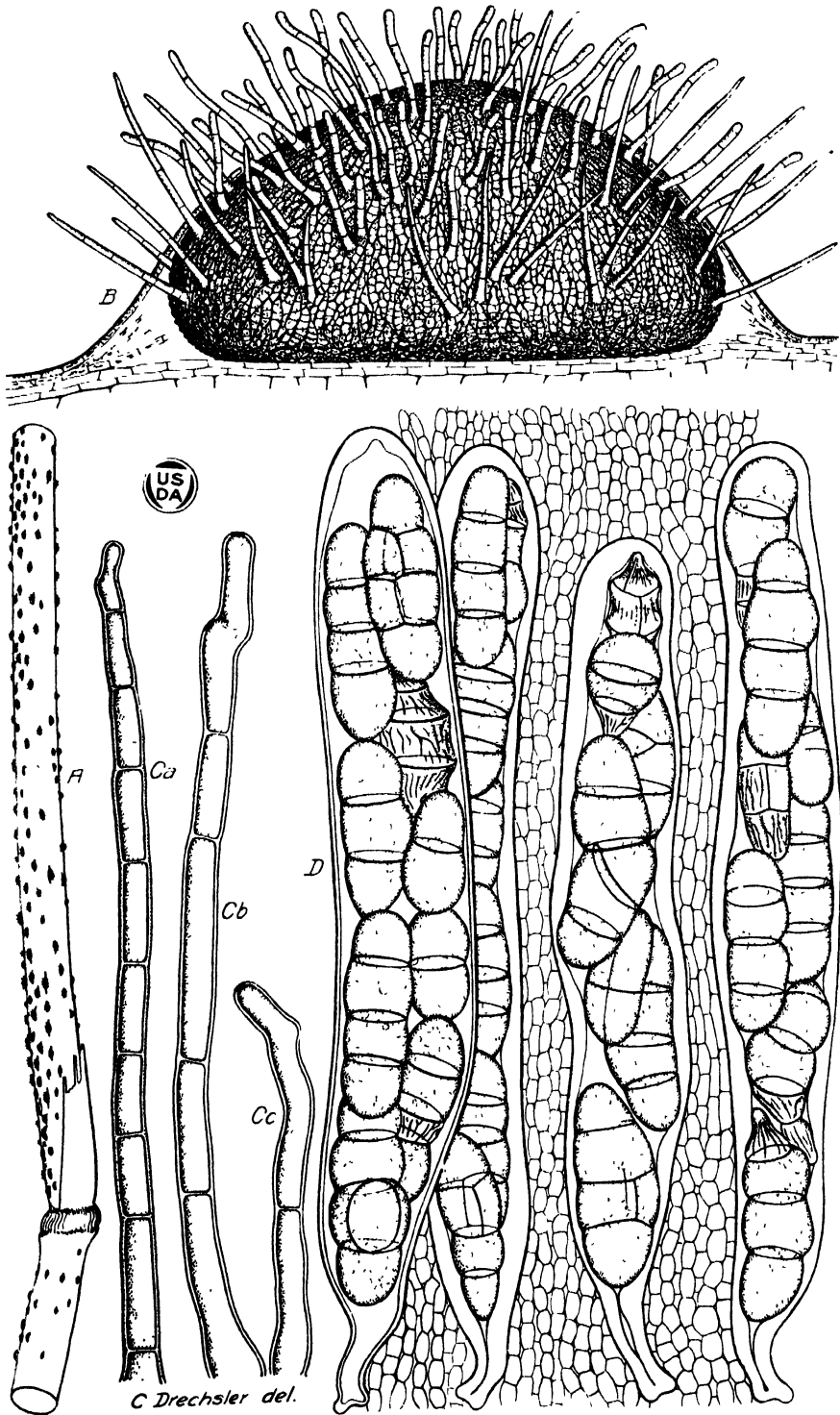
Da-d - Conidia from diseased barley leaf germinating in tap water, showing proliferation of germ tubes from end and middle segments.  $\times 500$

Ea-d - Conidiophores emerging from stoma in group of 3, and singly from between adjacent epidermal cells.  $\times 500$

PLATE 3

*Pyrenophora teres*

- A. —Portion of barley culm as found occurring near Madison, Wis., April 15, 1919, showing numerous crumpled perithecia.  $\times 2$
- B. —Perithecium bearing pointed setae near base, and an abundance of flexuous conidiophores on upper surface.  $\times 115$
- Ca. —Seta from perithecium.  $\times 500$
- Cb-c. —Conidiophores from perithecium.  $> \times 500$ .
- D. —Four asexual asci showing imperfect development of ascospores, and pseudoparenchyma surrounding them.  $\times 500$





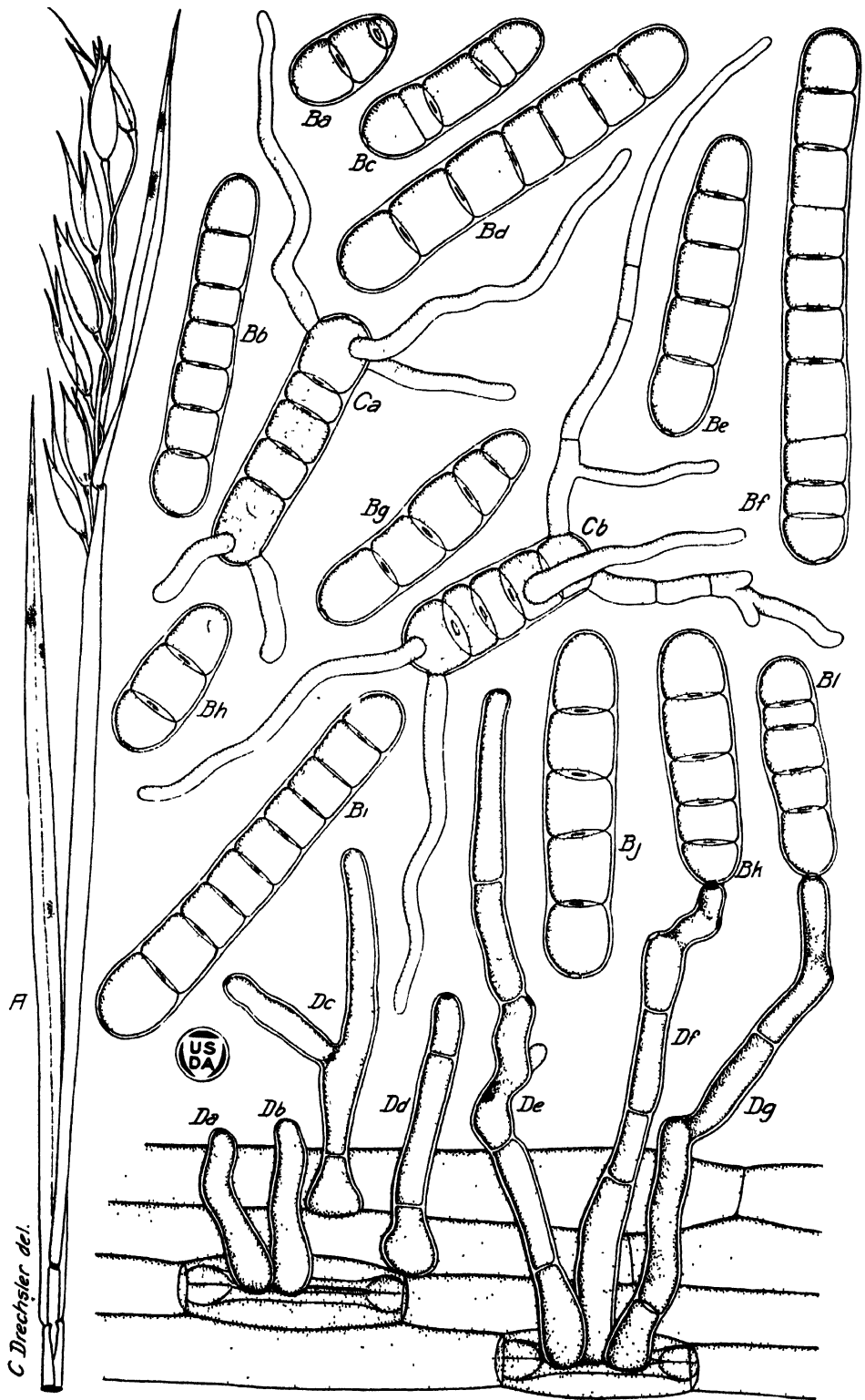


PLATE 4

*Helminthosporium avenae*

A—Portion of oat plant showing a number of discolored areas on upper leaves due to attack by *H. avenae*  $\times 4$

Ba-f—Spores of *H. avenae* from diseased oat leaf showing variation in size, shape, and septation  $\times 500$

Ca-b—Spores from diseased oat leaf, germinating in water showing production of germ tubes from end and middle segments  $\times 500$

Da-g—Conidiophores emerging singly or in groups of 2 or 3, from stomata or between contiguous epidermal cells. *Dc* and *De* exhibit type of branching occurring occasionally in this species  $\times 500$

PLATE 5

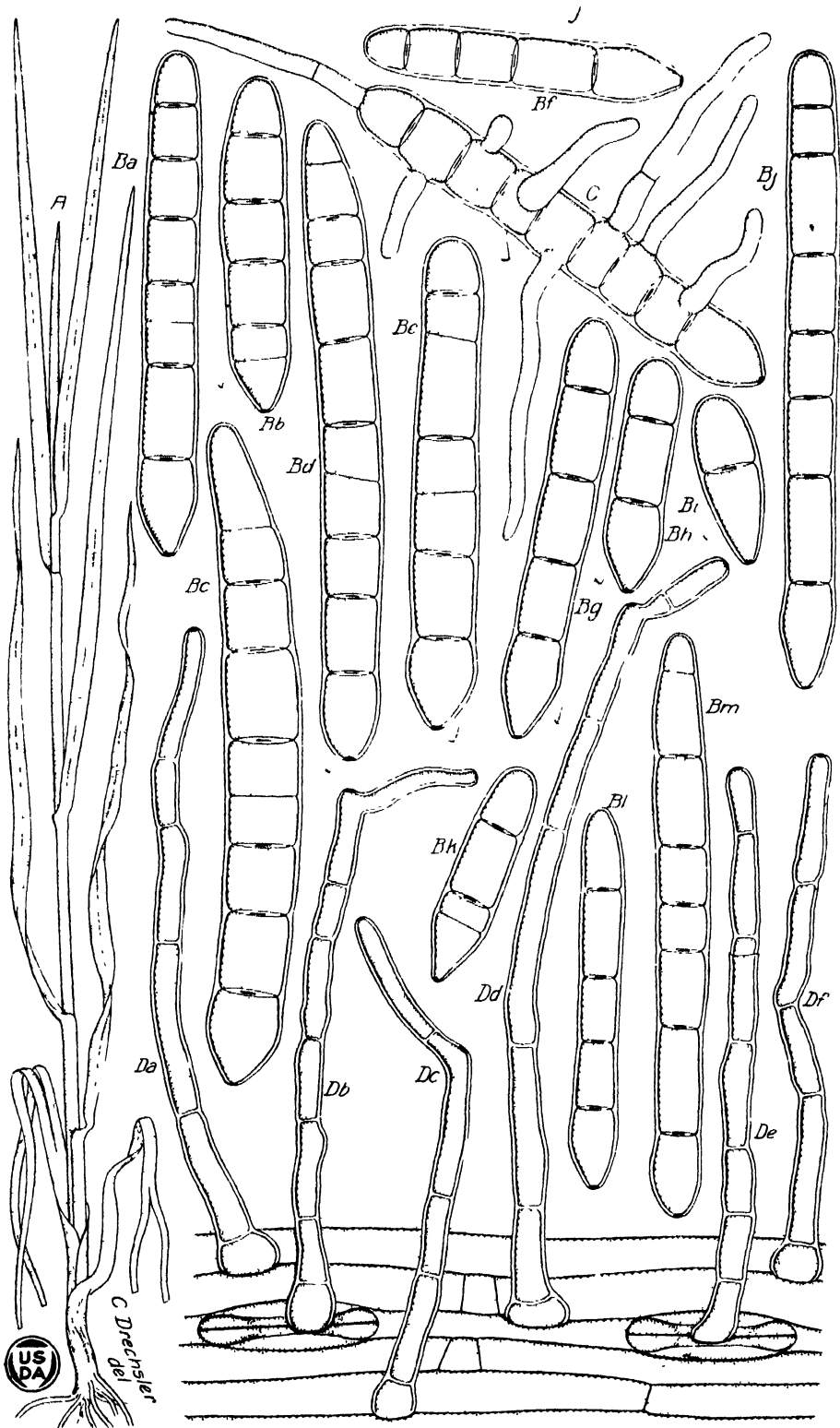
*Helmintosporium tritici-repentis*

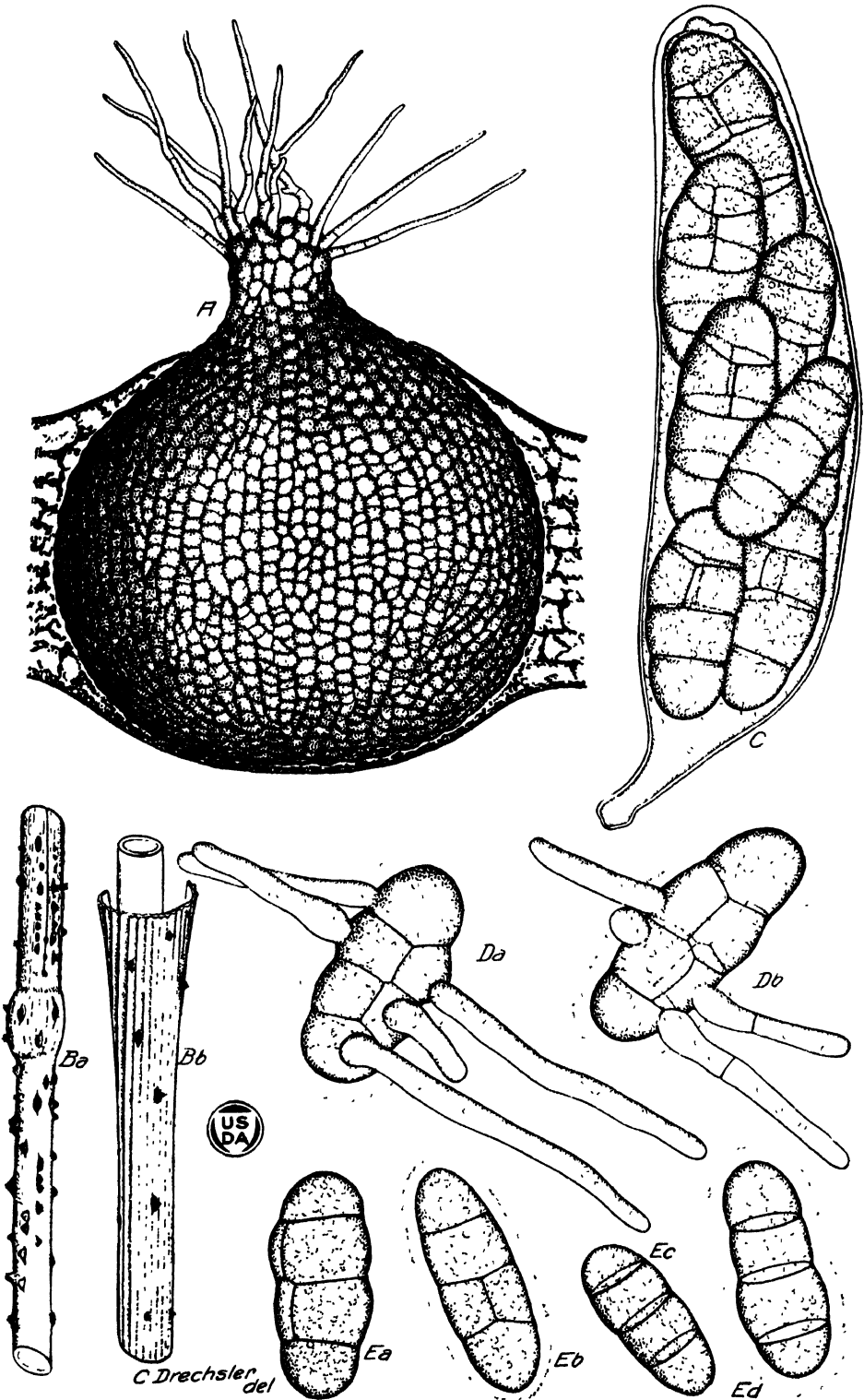
A.—Small plant of *Agropyron repens*, as found near Brooklyn, N. Y., July 12, 1920, showing four lower leaves withered and killed as result of attack of fungus, discoloration absent but microscopical examination revealing abundance of fructifications of *H. tritici-repentis* on three lowermost leaves  $\times 34$ .

Ba-m.—Conidia showing variation in size, shape, dimensions, and abundance of septation and the distinctive contour of the basal segment characteristic of the species  $\times 500$ .

C.—Conidium germinating normally in water mount by proliferation of germ tubes indiscriminately from nearly all segments  $\times 500$ .

Da f.—Conidiophores showing mode of emergence from stomata and from between adjacent epidermal cells  $\times 500$ .





## PLATE 6

### *Pycnophora tritici repens*

A Peritheecium of *Pycnophora tritici repens* collected on decaying leaves of *Aquopyron repens* near Madison, Wis., early in April, 1910, showing well-defined ostiolar beak bearing a number of setae at the tip, the latter being more or less curved, often several times septate, occasionally branching, and tapering perceptibly from base to distal end  $\times 175$

Ba-b Portions of old stem of *Aquopyron repens*, showing numerous erumpent perithecia appearing on denuded stalk (Ba), and less numerous perithecia on leaf sheath (Bb)  $\times 5$

C Ascus freshly dissected from peritheecium before undergoing swelling preliminary to spore discharge  $\times 500$

Da-b Ascospores germinating by production of germ tubes from the greater number of their segments, showing also the enveloping gelatinous layer  $\times 500$

Ea-d Ascospores showing variation in size and septation, as well as presence and absence of enveloping gelatinous layer  $\times 500$

PLATE 7

*Helminthosporium catenarium*

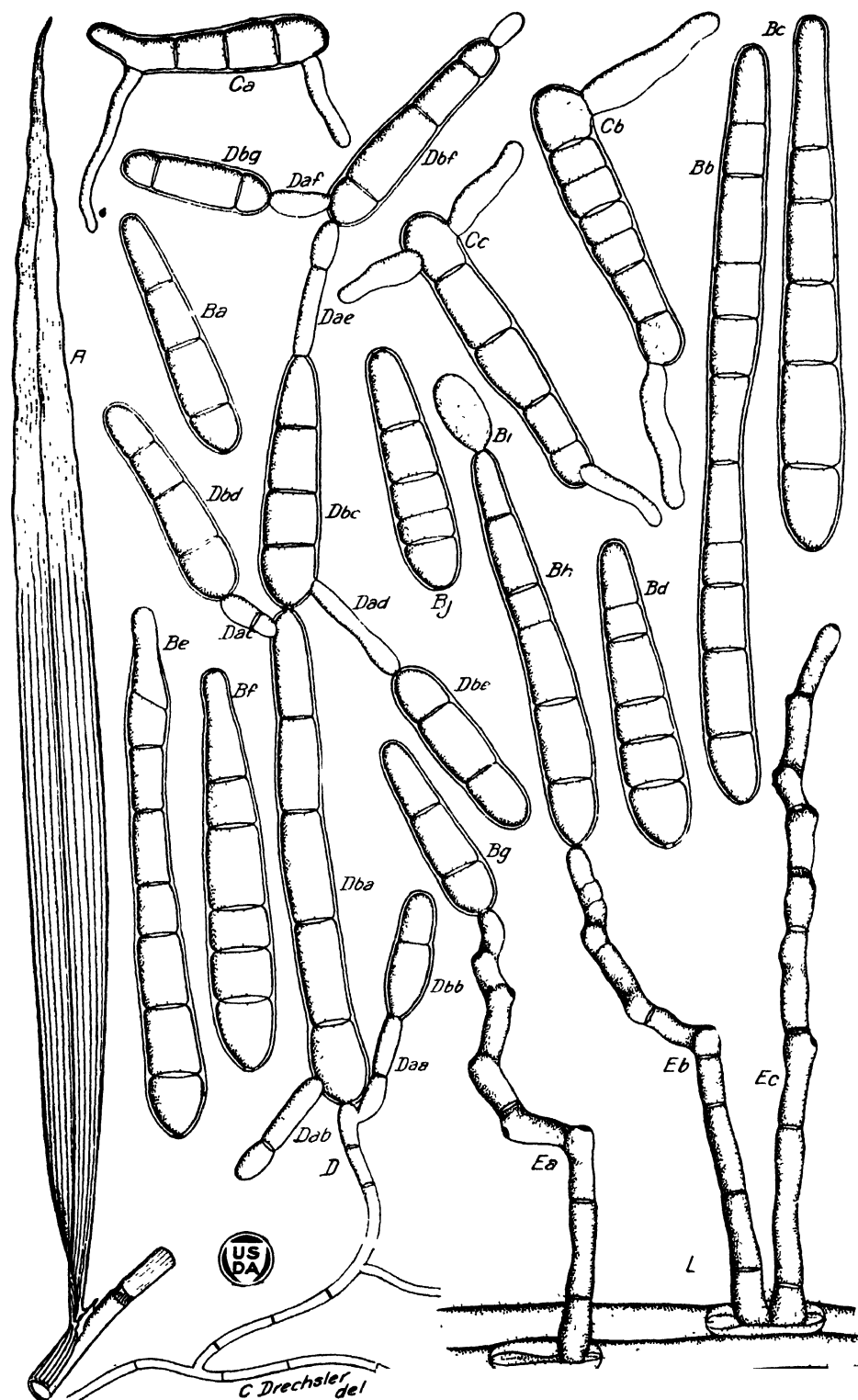
A Leaf of *Cinna arundinacea*, the proximal portion healthy, the distal portion entirely withered as result of attack by *H. catenarium*  $\times 3\frac{1}{4}$

Ba-i —Spores of *Helminthosporium catenarium* from material collected on host, showing variation in size, shape, and septation, *Ba, d, g, j*, short spores, straight and tapering toward tip *Bb*, long spore with median constriction, *Bc, f*, long spores showing constricted apical prolongation, with apical scar marking point of attachment of secondary spore, *Be*, long spore, irregular in diameter, growing out at apex to produce a secondary spore, *Bh*, primary spore with young secondary spore *Bi* attached at apex  $\times 500$ .

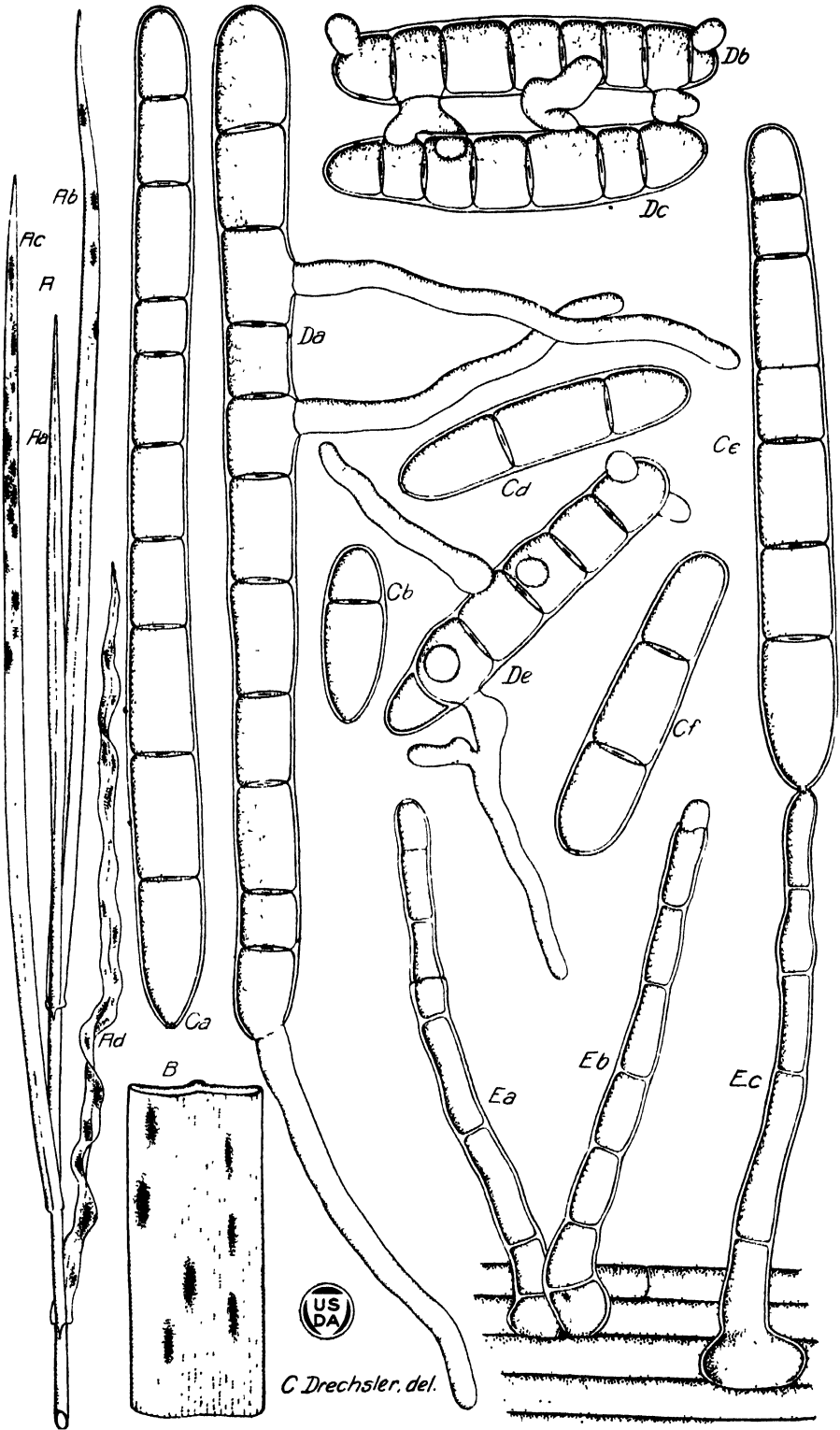
Ca-c —Spores from material collected on host, germinating in tap water by production of germ tubes from basal and apical segments  $\times 500$

D Conidial fructification in 20-day-old corn-meal agar culture, consisting of sporophoric segments *Daa-af*, and spores *Dba bg*  $\times 500$

Ea-e —Sporophores from material collected in field, emerging from stomata (epidermis and stomata considerably distorted as result of withering)  $\times 500$







# PLATE 8

## *Helminthosporium bromi*

A Portion of plant of *Bromus inermis*, showing youngest leaf (1a) healthy, the second (1b), and third (1c) bearing a number of spots, and the fourth (1d) largely withered as a result of attack by *Helminthosporium bromi* <sup>4</sup>

B Portion of diseased leaf of *Bromus inermis*, showing etiolated areas surrounding dark spots, the discoloration in the latter being most intense in the center and fading out toward the periphery

Ca f Conidia from diseased leaf, showing variation in size, shape, and septation  
· 500

D a c Conidia germinating in tap water by the production of germ tubes indifferently from end and middle segments The spores Db and Dc germinated lying in contact, the resulting germ tubes anastomosing immediately after their proliferation  
· 500

E a c Conidiophores of *H. bromi* emerging between adjacent epidermal cells of host  
· 500

PLATE 9

*Pycnophora bromi*

A --Old leaf of *Bromus inermis* of growth of preceding season as collected near Madison, Wis., May 1, 1920, showing scattered mature perithecia of *P. bromi*  $\times 34$

B --Perithecium of *P. bromi*, showing wide ostiolar beak, with scattering setae  $\times 155$

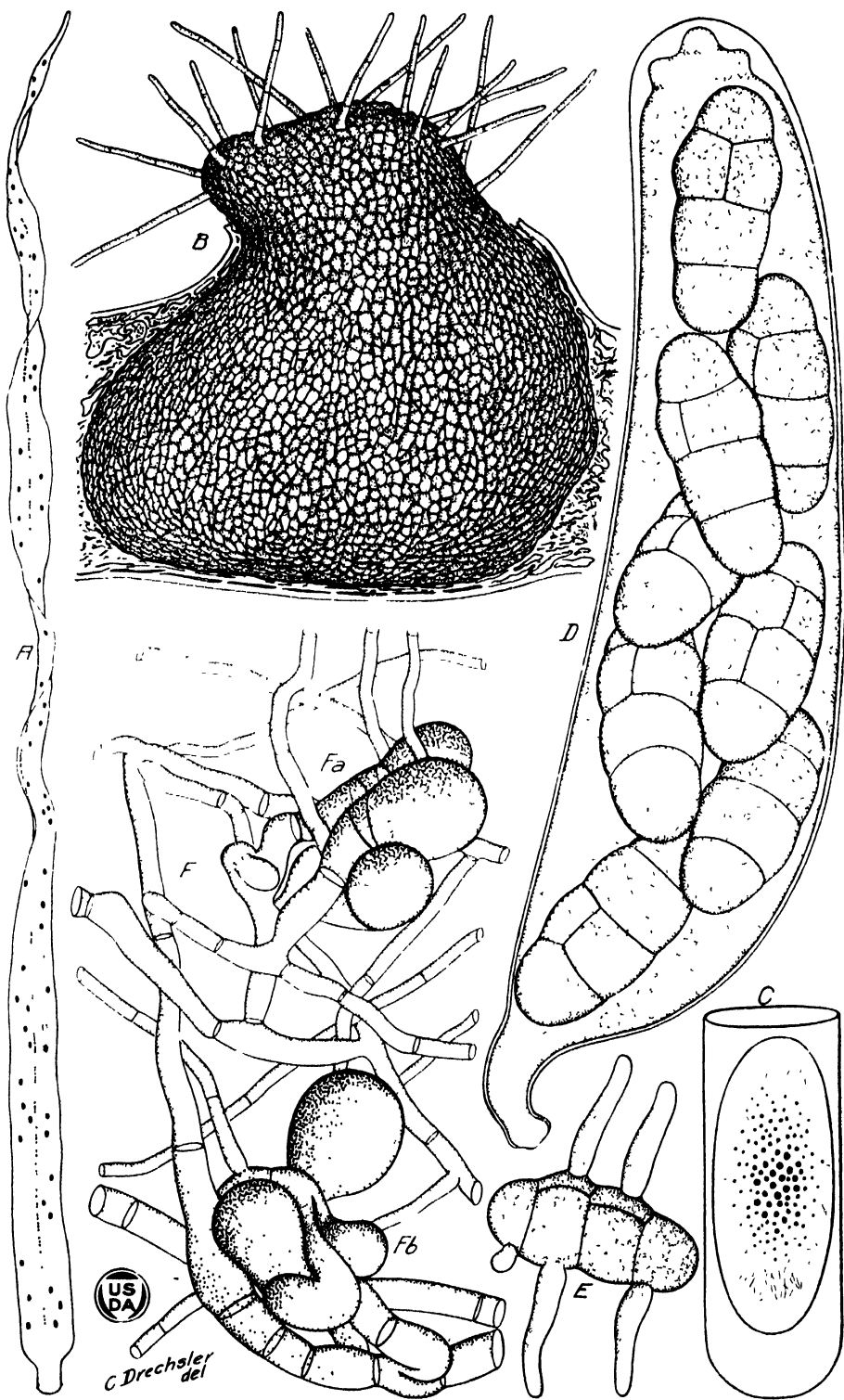
C --Twenty five day old potato agar culture of *P. bromi*, showing sclerotia partly covered with white mat of aerial mycelium

D --Ascus of *P. bromi* after some swelling due to absorption of water, preliminary to spore discharge  $\times 500$

E -- Ascospore of *P. bromi* germinating in water

*Pycnophora teres*

F.--Submerged mycelium of *P. teres* from 5-day old water agar culture, showing relatively small complexes of inflated hyphal segments  $\times 500$



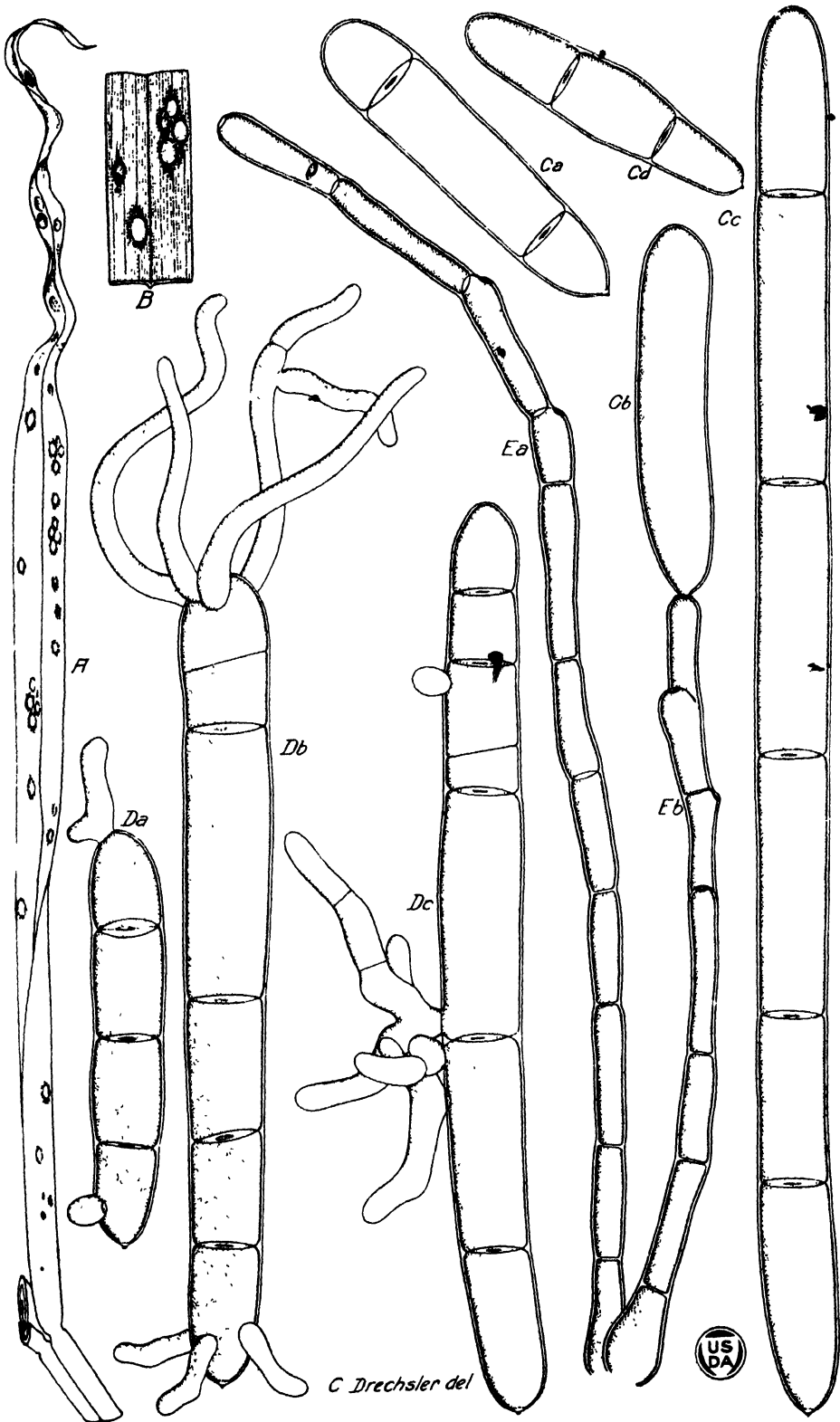


PLATE 10

*Helminthosporium giganteum*

A Leaf of *Eleusine indica* attacked by *H. giganteum*, showing the presence of numerous "eye spot" lesions and the withering of the tip as a result of the disease. × 1

B Portion of leaf of *Eleusine indica*, showing eye spot lesions in various stages of development. × 2

Ca-d Normal conidia from leaf of *Eleusine indica*, showing variation in size and shape and presence of conical protuberance at base. The conidium (c) represents approximately the maximum length attained by spores of the fungus. × 500

Da-e Conidia from diseased leaf germinating in water by the production of germ tubes singly (Da), or in groups from the end cells (Db), or from intermediate cells (Dc). × 500

Ea-b Conidiophores from diseased leaf of *Eleusine indica* showing enlarged base and spacing of septa and of scars. × 500

PLATE 11

*Helminthosporium dictyoides*

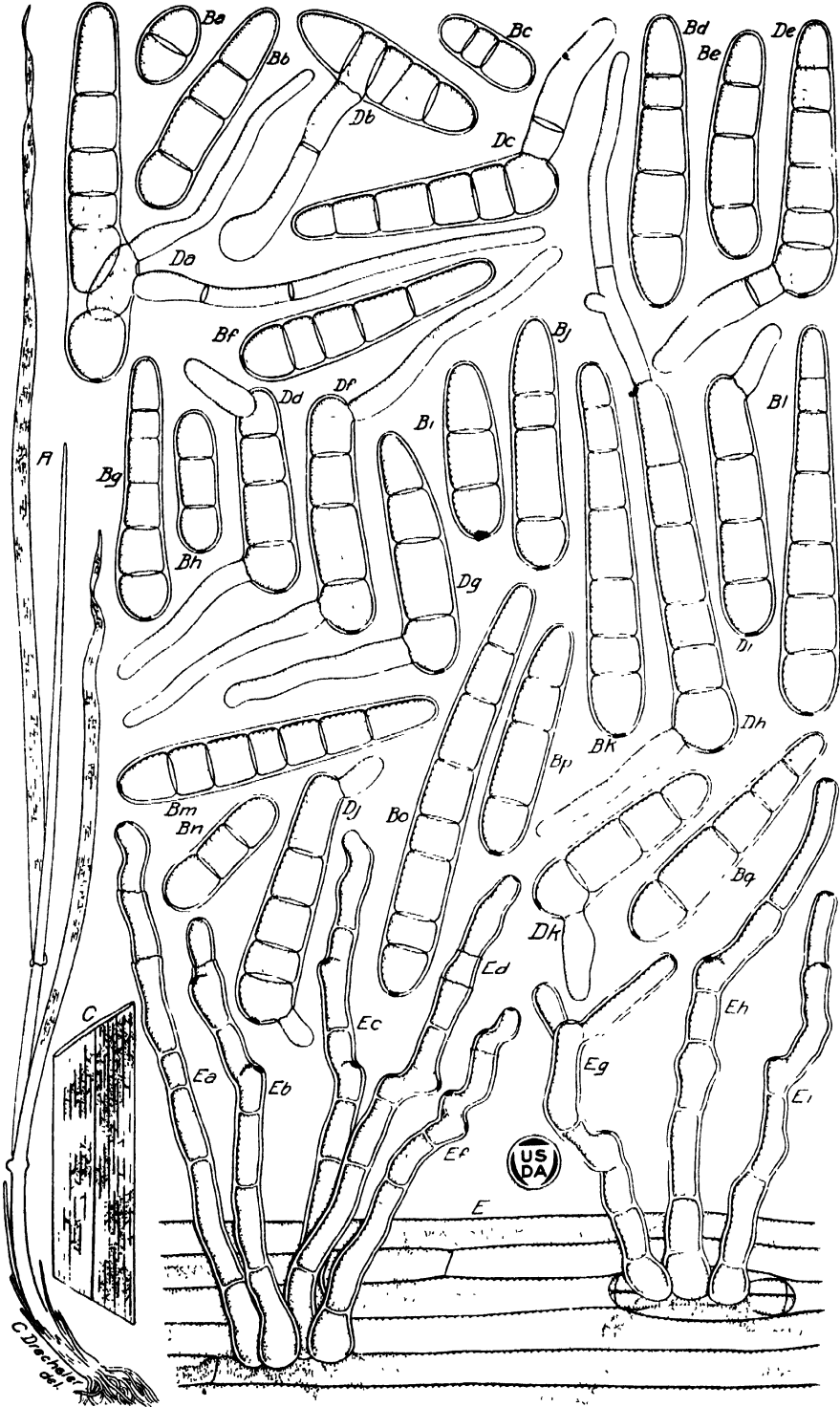
A - Young plant of *Festuca elatior*, showing numerous net-blotch lesions on the two older leaves, the lowermost one beginning to wither at the tip.  $\times \frac{3}{4}$

Ba-c - Conidia from leaf of *Festuca elatior*, showing variation in size, shape, and septation.  $\times 500$

C - Part of leaf of *Festuca elatior*, showing reticulate pattern of dark longitudinal and transverse streaks within diffusely discolored areas.  $\times 3$

Da-k - Conidia from leaf of *Festuca elatior*, germinating in water by the production of lateral or oblique germ tubes, usually from the end segments, and less frequently from an intermediate segment (Da, b).  $\times 500$

Ea-1 - Conidiophores of *H. dictyoides*, one group (Ja-f) emerging from between adjacent epidermal cells, the other group (Eg-1) emerging from a stoma. Branching like that shown in Eg is relatively infrequent. Hyphae within the tissue of the leaf, visible in glycerine preparations stained with eosin are indicated by heavier stippling.  $\times 500$





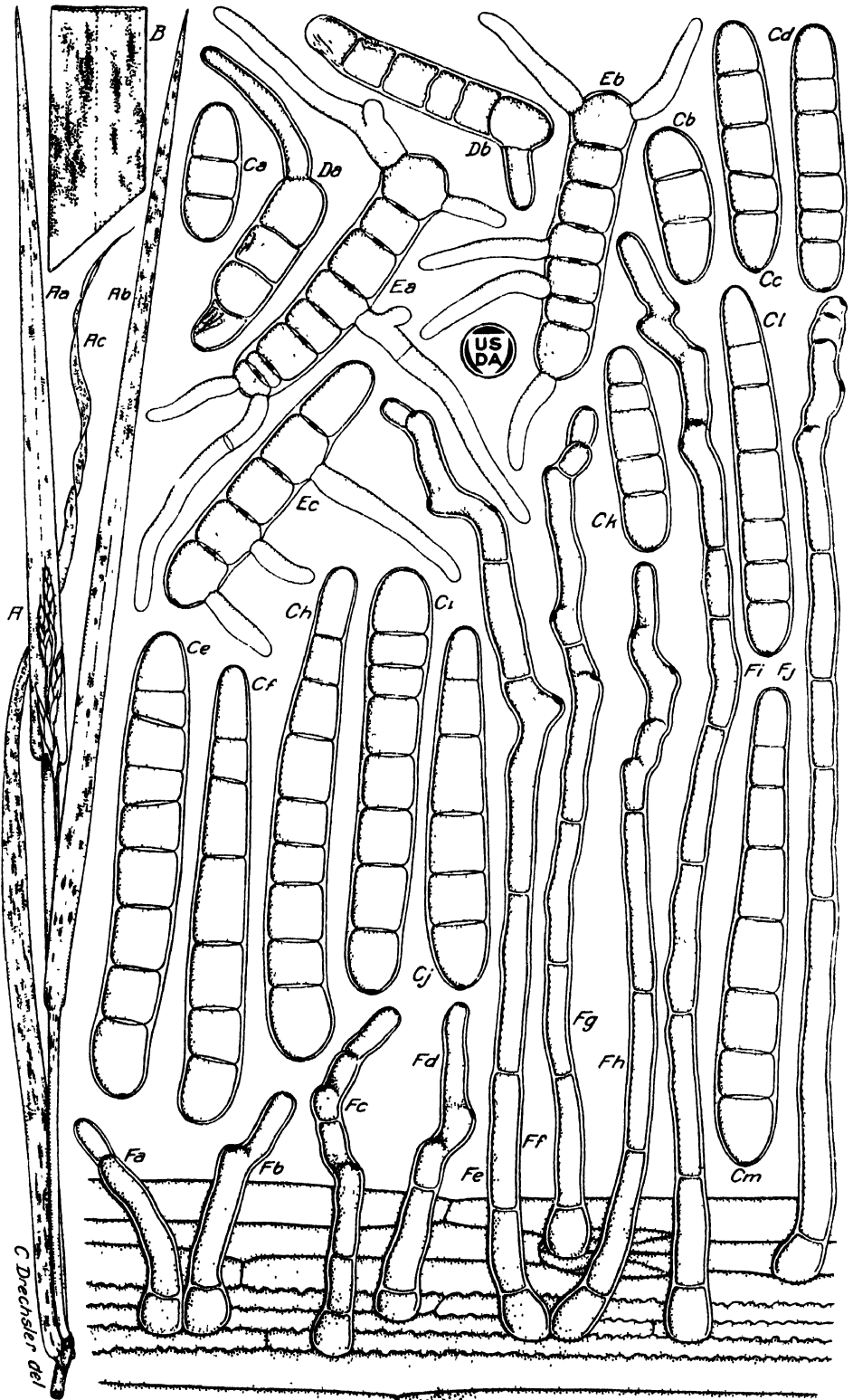


PLATE 12

*Helminthosporium viciae*

A—Portion of plant of *Lolium multiflorum* attacked by *Helminthosporium viciae*, the upper leaf *Aa* showing relatively few discolored spots, the next leaf *Ab* showing a greater number, and the third, which has begun to wither at the tip, showing a large number of spots × 1

B—Portion of diseased leaf, showing spots somewhat magnified × 4

Ca-m. Conidia from leaf of *Lolium multiflorum*, showing variation in size, shape, and abundance of septation × 500

Da-h. Conidia from leaf of *Lolium multiflorum* that have germinated by the production of a short sporophoric process on which has been produced a single secondary conidium × 500

Ea-c. Conidia from leaf of *Lolium multiflorum* germinating in water by the production of germ tubes from both end and intermediate segments × 500

Fa-j. Conidiophores from leaf of *Lolium multiflorum*, showing variation in size, and mode of emergence from stoma or more abundantly from between epidermal cells on vascular ridges × 500

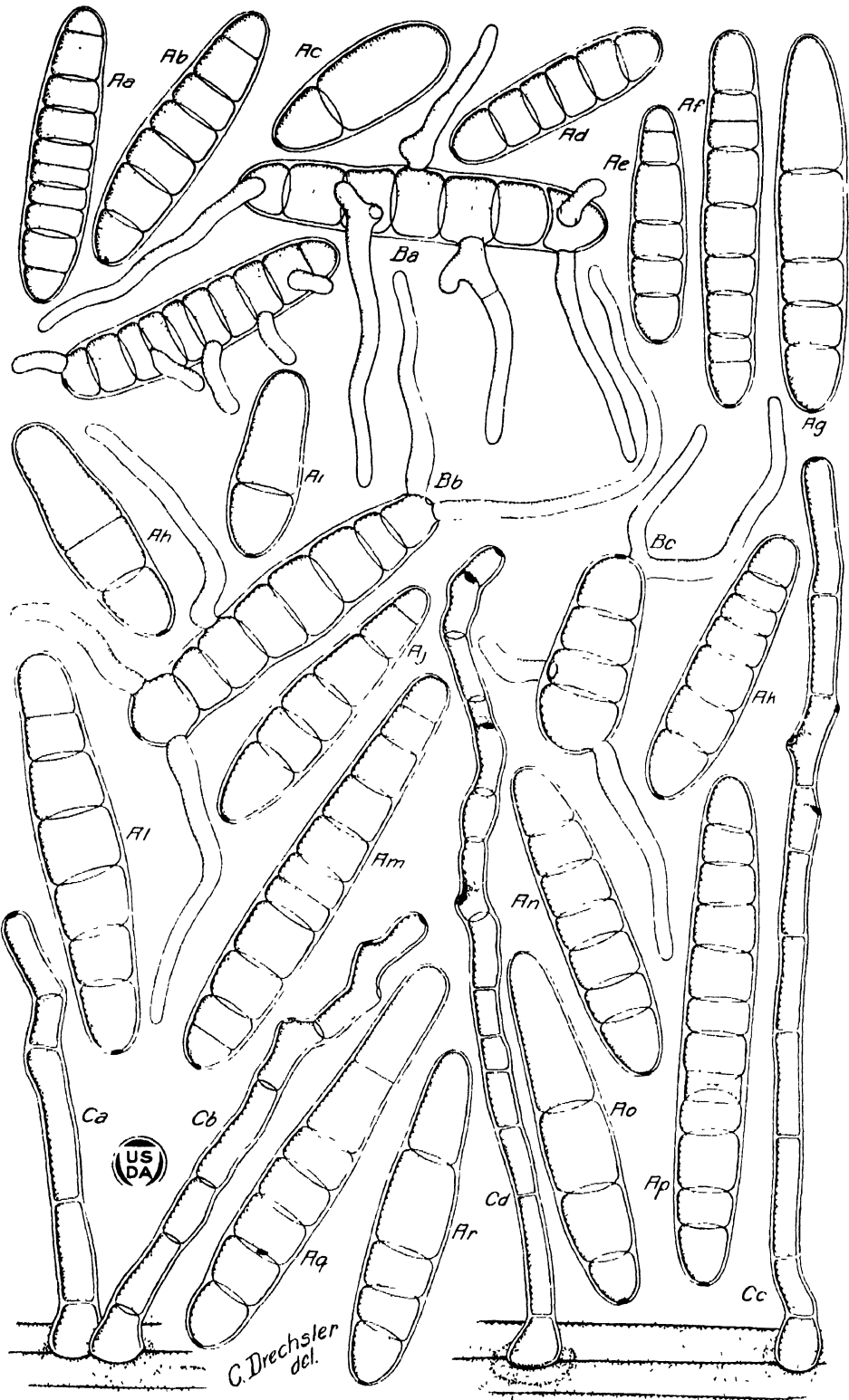
PLATE 13

*Helminthosporium stenacrum*

Aa-r Conidia from leaf of *Agrostis stolonifera* showing variation in size, shape, and septation. In Aq, h, i, l, o, q, r, distal portion produced as a somewhat attenuated prolongation, in which septa are absent or present in smaller numbers than in unmodified region.  $\times 500$ .

Ba c --Conidia from leaf of *Agrostis stolonifera* germinating in water by the production of germ tubes from both middle and end segments.  $\times 500$

Ca b Conidiophores of *H. stenacrum* showing variation in size and mode of emergence singly or in pairs from between adjacent epidermal cells.  $\times 500$



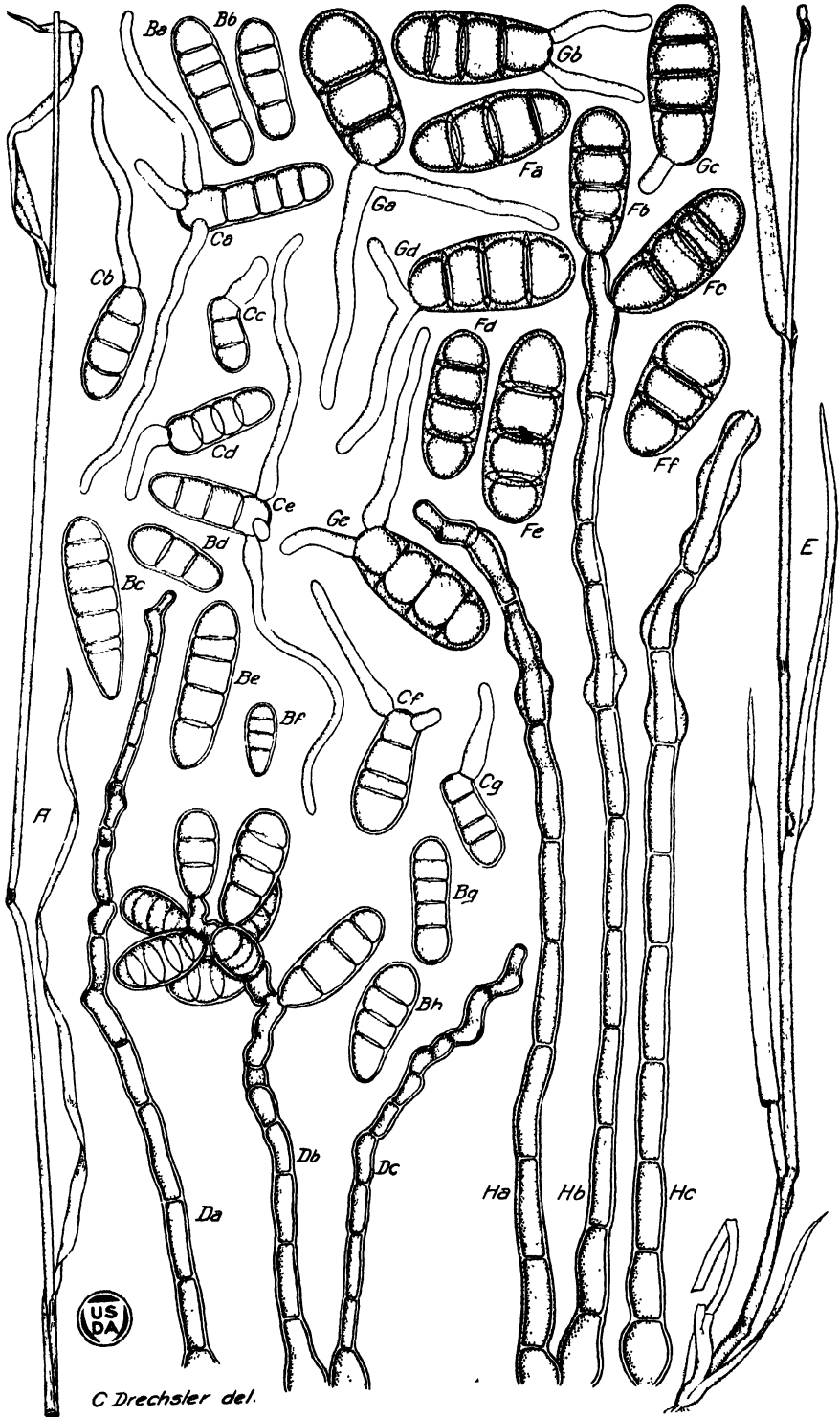


PLATE 14

*Helminthosporium dematioides*

A—Portion of plant of *Anthoxanthum odoratum*, with leaves withered and bearing fructifications of *H. dematioides*  $\times 4$ .

Ba-h—Conidia from withered leaf of *Anthoxanthum odoratum*, showing variation in size, shape, and septation  $\times 500$ .

Ca-g—Conidia from withered leaf of *Anthoxanthum odoratum* germinating in water, atypically g(a, c-a) by the production of one to three germ tubes from the basal segment and typically (c-b) by production of one germ tube from apex, owing to death of basal segment  $\times 500$ .

Da-e—Conidiophores of *H. dematioides* from leaf of *Anthoxanthum odoratum*.  $\times 500$ .

*Helminthosporium triseptatum*

E—Portion of plant of *Notholcus lanatus* with lower leaves withered and bearing fructifications of *H. triseptatum*  $\times 4$ .

Fa-f—Conidia from leaf of *Notholcus lanatus* showing variation in size, shape, and septation  $\times 500$ .

Ga-e—Conidia from leaf of *Notholcus lanatus* germinating in water by the production of one or two germ tubes from basal end  $\times 500$ .

Ha-e—Conidiophores from leaf of *Notholcus lanatus* showing local ring like thickenings of wall immediately below points of attachment of successive spores  $\times 500$ .

PLATE 15

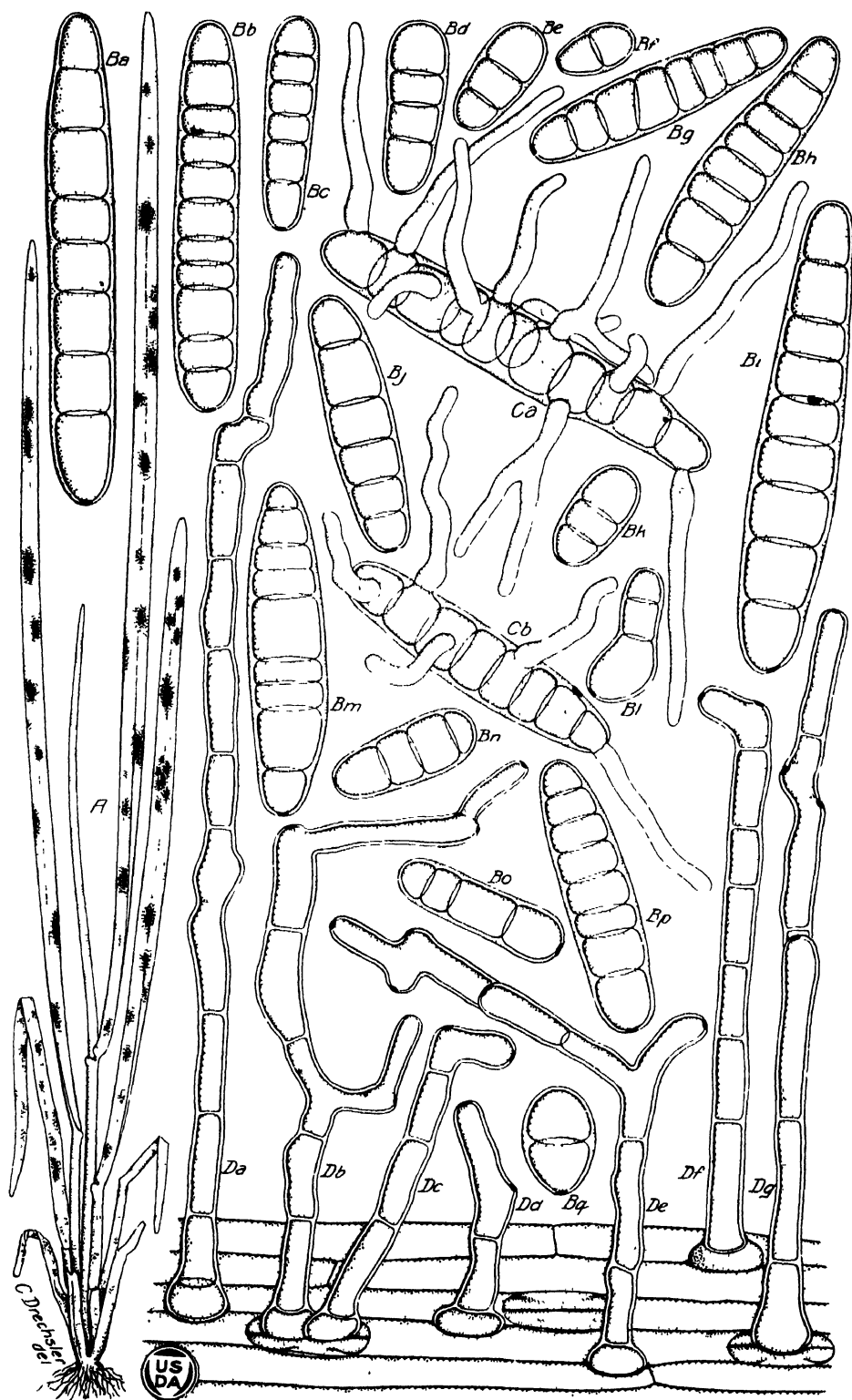
*Helminthosporium vagans*

A *Poa pratensis* with leaves abundantly spotted as result of numerous local infection by *H. vagans*  $\times 14$  Drawn from material collected in Brooklyn, N. Y. August, 1920

Ba, c Conidia produced on leaf of *Poa pratensis*, showing variation in size, shape and septation  $\times 500$

Ca, b Conidia produced on leaf of *Poa pratensis*, germinating in water by production of germ tubes indiscriminately from end and middle segments  $\times 500$

Da-g Conidiophores of *H. vagans*, showing variation in dimension, shape, and septation, emergence singly or in pairs from stomata or between epidermal cells, and occasional irregular branching characteristic of the species  $\times 500$ .





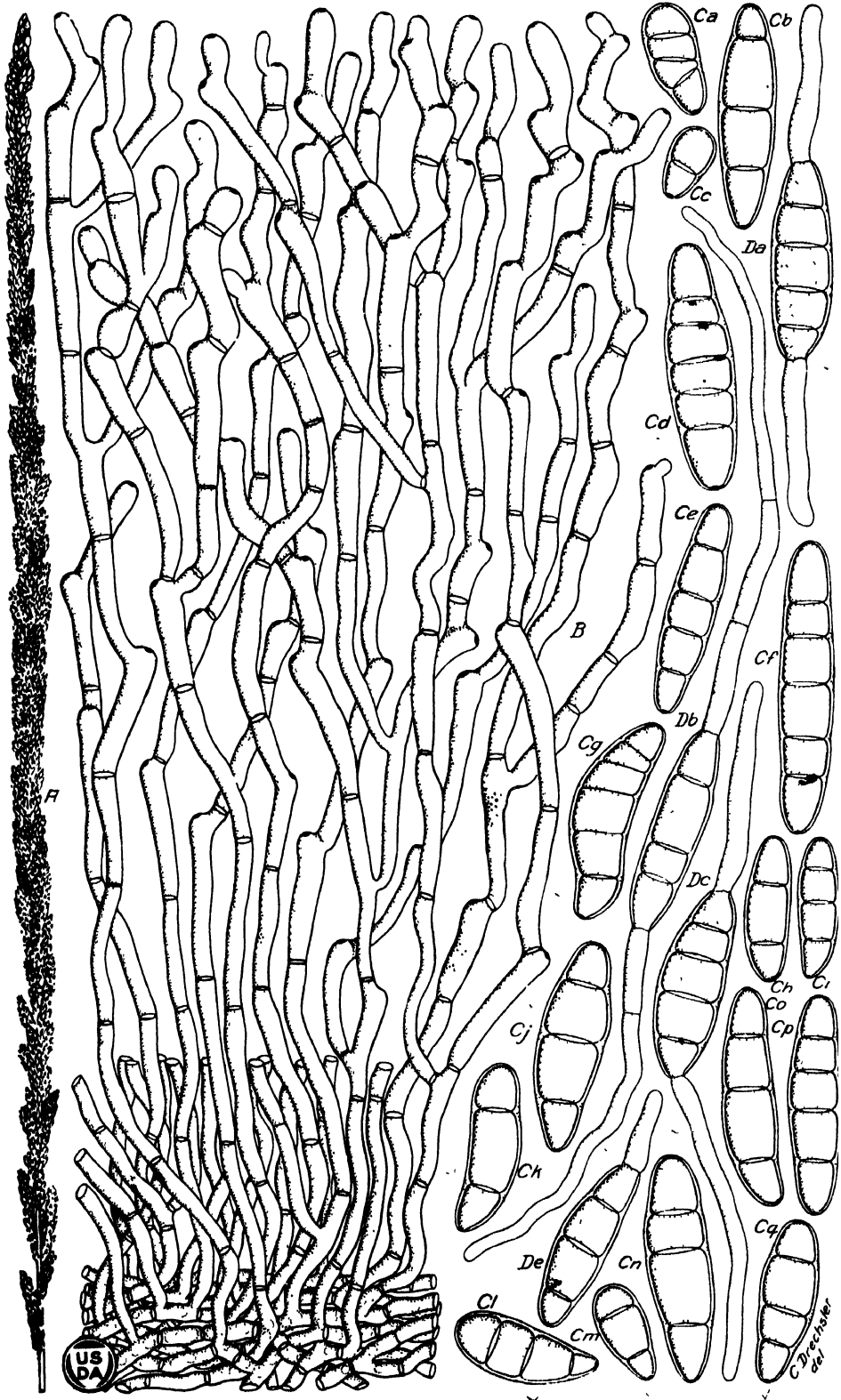


PLATE 16

*Helminthosporium ravenehi*

A - Inflorescence of *Sporobolus indicus*, the lighter portions healthy, the darker portions covered with the conidial fructifications of *H. ravenehi*  $\times 34$  Drawn from material collected at Wauchula, Florida, April 15, 1921

B - Conidiophores of *H. ravenehi*, showing origin from interwoven hyphae on superficial layers of floral parts of host, crowded condition, branching habit, irregularity in diameter, and scars marking points of attachment of spores  $\times 500$

Ca - q - Conidia showing variation in shape, size, and septation  $\times 500$

Da - e - Conidia germinating in water by production of one or more, typically two, polar germ tubes, one from each end cell  $\times 500$

PLATE 17

*Helminthosporium sativum*

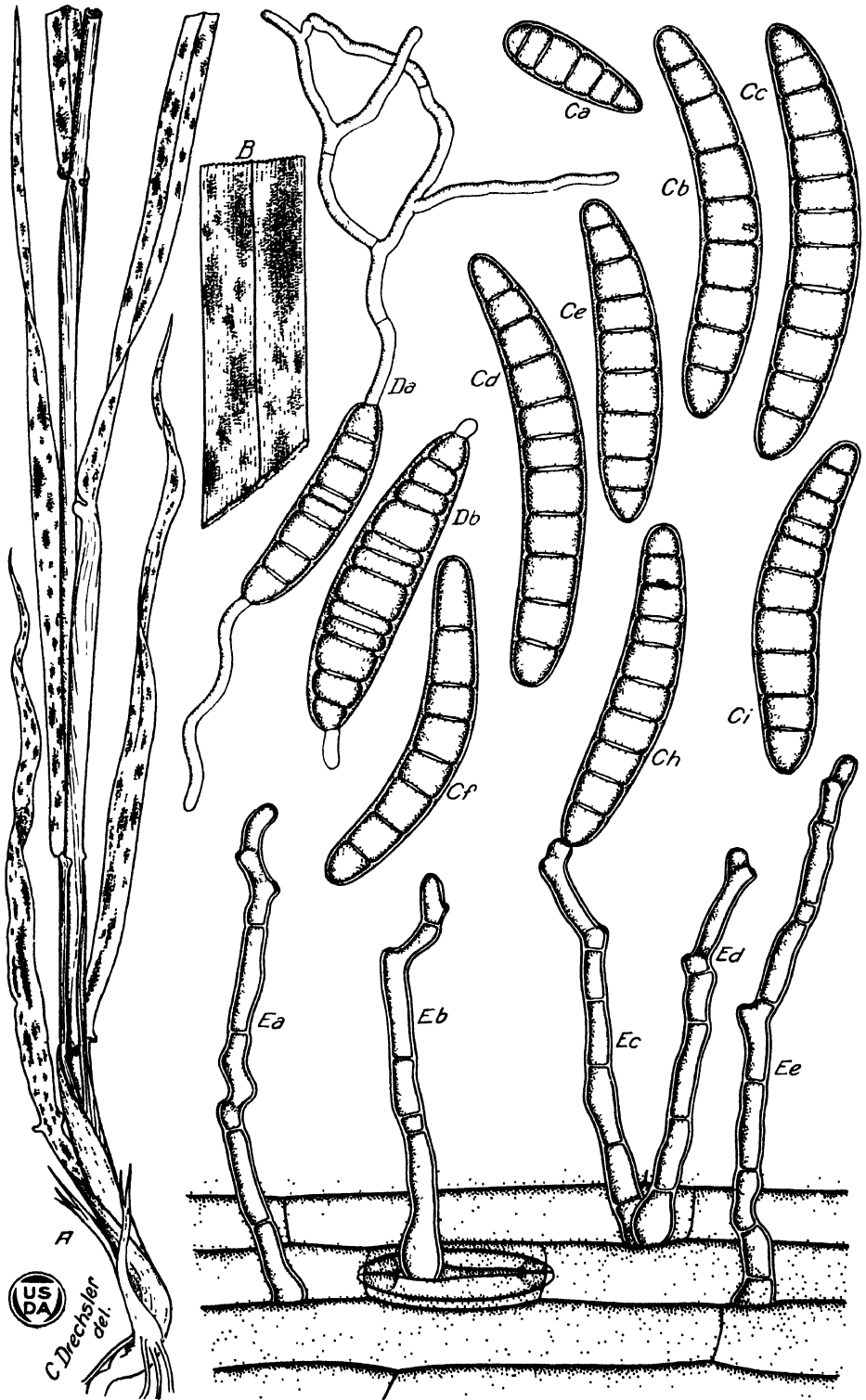
A --Portion of barley affected with spot-blotch, showing abundance of foliar lesions and withering of lower leaves  $\times 34$  Drawn from material collected at Madison, Wis., July 22, 1910

B - Portion of barley leaf showing numerous discolored areas due to infection with *H. sativum*  $\times 2$

C -Conidia produced on diseased barley, showing characteristic shape, and variation in size and septation  $\times 500$

D --Conidia of *H. sativum* from diseased barley leaf germinating in water by the production of two polar germ tubes.  $\times 500$

Eane -Conidiophores of *H. sativum* emerging from stoma and between epidermal cells of barley leaf  $\times 500$



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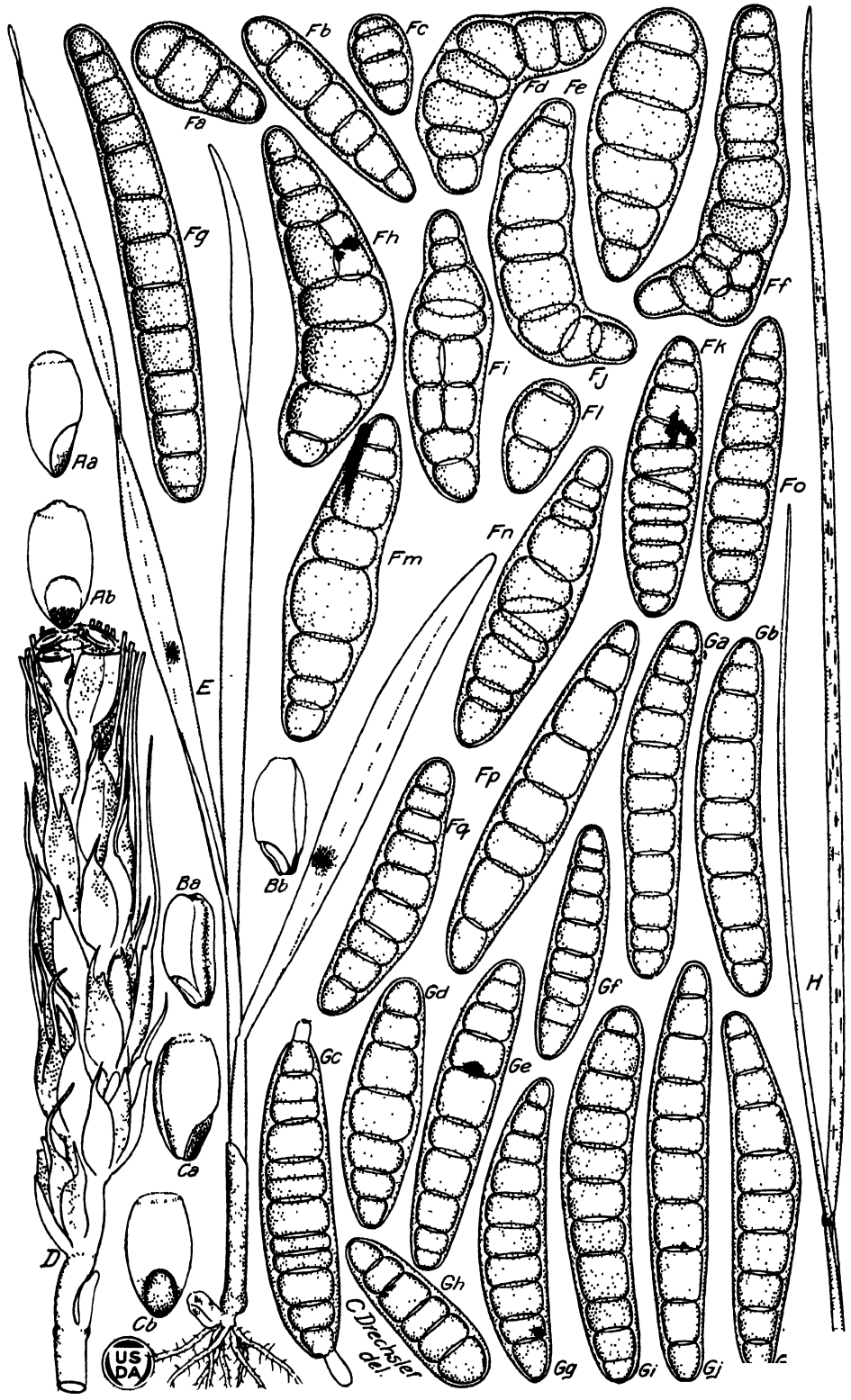


PLATE 18

*Helminthosporium sativum*

Aa-b. Kernels of wheat affected with "black point," lateral and dorsal views.  
×3.

Ba-b. Wheat kernels attacked by *H. sativum*, showing extensive discoloration of embryo and ventral suture, median longitudinal section and lateral view. ×3.

Ca-b. Wheat kernels showing severe discoloration in region of embryo, lateral and dorsal views. ×3.

D. Portion of mature wheat head, showing grayish efflorescence on glumes, composed of fructifications of *H. sativum*. ×3½.

E. Wheat seedling grown from "black pointed" kernel showing moderately severe infection with *H. sativum*, discoloration of the basal sheath and two foliar spots. ×3¼.

Fa-q. Conidia of *H. sativum* produced on wheat head, showing atypical irregularities in shape, size, and septation. ×500.

Ga-k. Typical conidia produced on infected leaves of *Agropyron repens*, illustrating variation in shape, size, and septation. ×500.

H. Upper leaves of *Agropyron repens* attacked by *H. sativum*, showing abundance of small elongated foliar spots. ×3¼.

PLATE 19

*Helminthosporium sativum*

A.—Denuded conidiophore from 50 day old cornmeal agar culture originally isolated from barley leaf, showing numerous scars marking places of attachment of spores and branched, contorted, distal portion.  $\times 500$

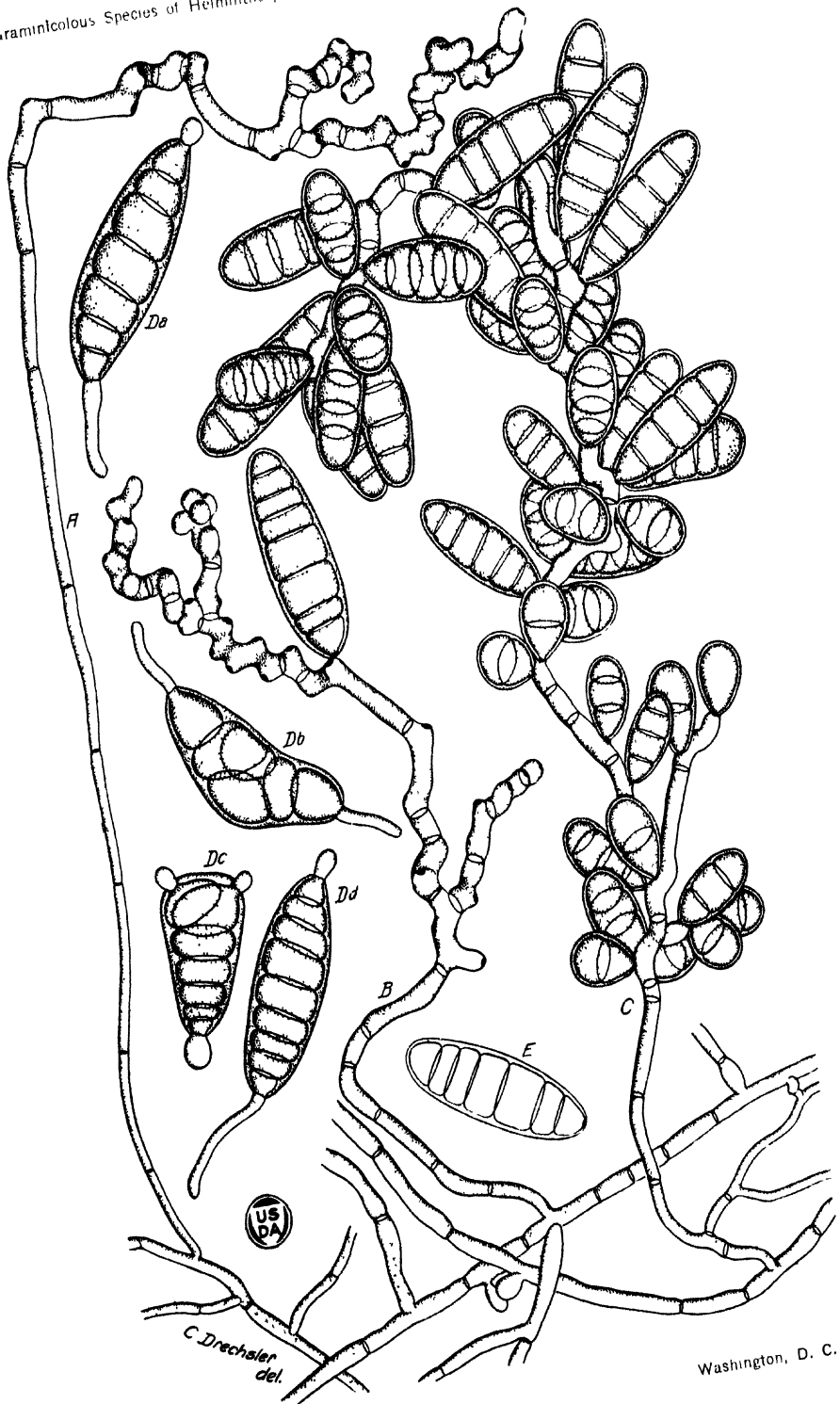
B.—Same as A, but shorter and bearing several branches.  $\times 500$

C.—Same as A, but showing approximately 50 conidia attached *in situ*. Note the variation in size of the spores from small subglobose non-septate forms borne mostly on the proximal portion of the conidiophore to the larger, many septate ellipsoidal specimens on the distal portion.  $\times 500$

Da-d - Conidia produced in cornmeal agar culture, germinating in water. Typical germination by the production of two polar germ tubes is represented in Da, b; atypical germination by the production of three germ tubes in Dc. In Db is illustrated also marked irregularity in septation frequent in conidia produced on artificial media.  $\times 500$ .

E.—Conidium produced on cornmeal agar showing position of hilum, thickness of peripheral wall, and shape characteristic of spores produced on artificial media.  $\times 500$

Graminicolous Species of *Helminthosporium*





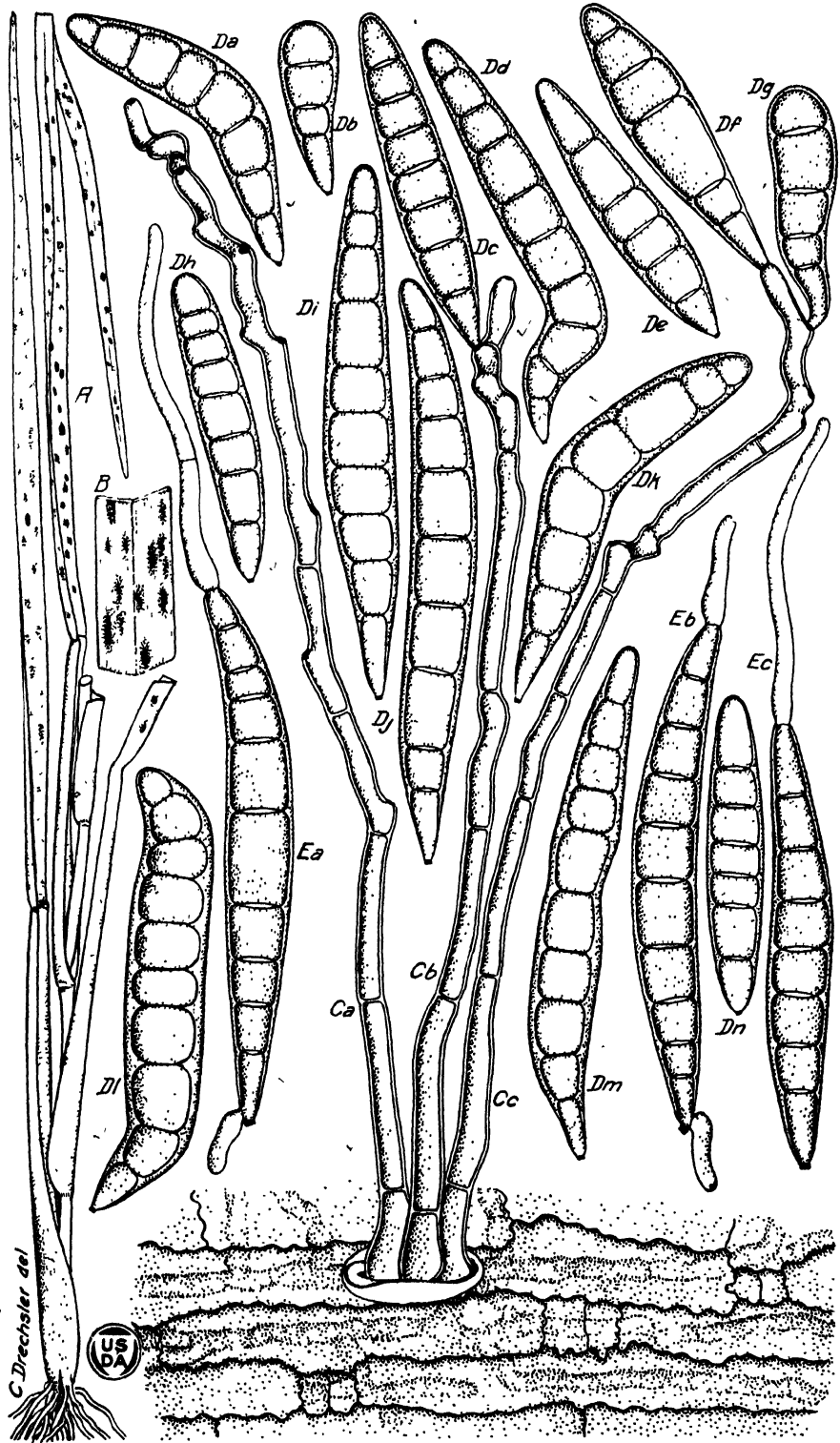


PLATE 20

*Helminthosporium monoceras*

A -- Portion of plant of *Echinochloa crusgalli* attacked by *H. monoceras* showing presence of spots on leaf blades and of diffused discoloration on basal sheaths  $\times 34$ .

B -- Portion of leaf blade of *Echinochloa crusgalli* attacked by *H. monoceras* showing variation in size of spots  $\times 34$ .

Ca-b -- Group of conidiophores emerging from stoma of host, showing also mycelium ramifying in mesophyll as revealed in glycerine preparations stained with eosin.  $\times 500$

Da-n -- Conidia from *Echinochloa crusgalli* collected at Port Washington, N. Y., September 20, 1920, showing variation in size, shape, and septation  $\times 500$

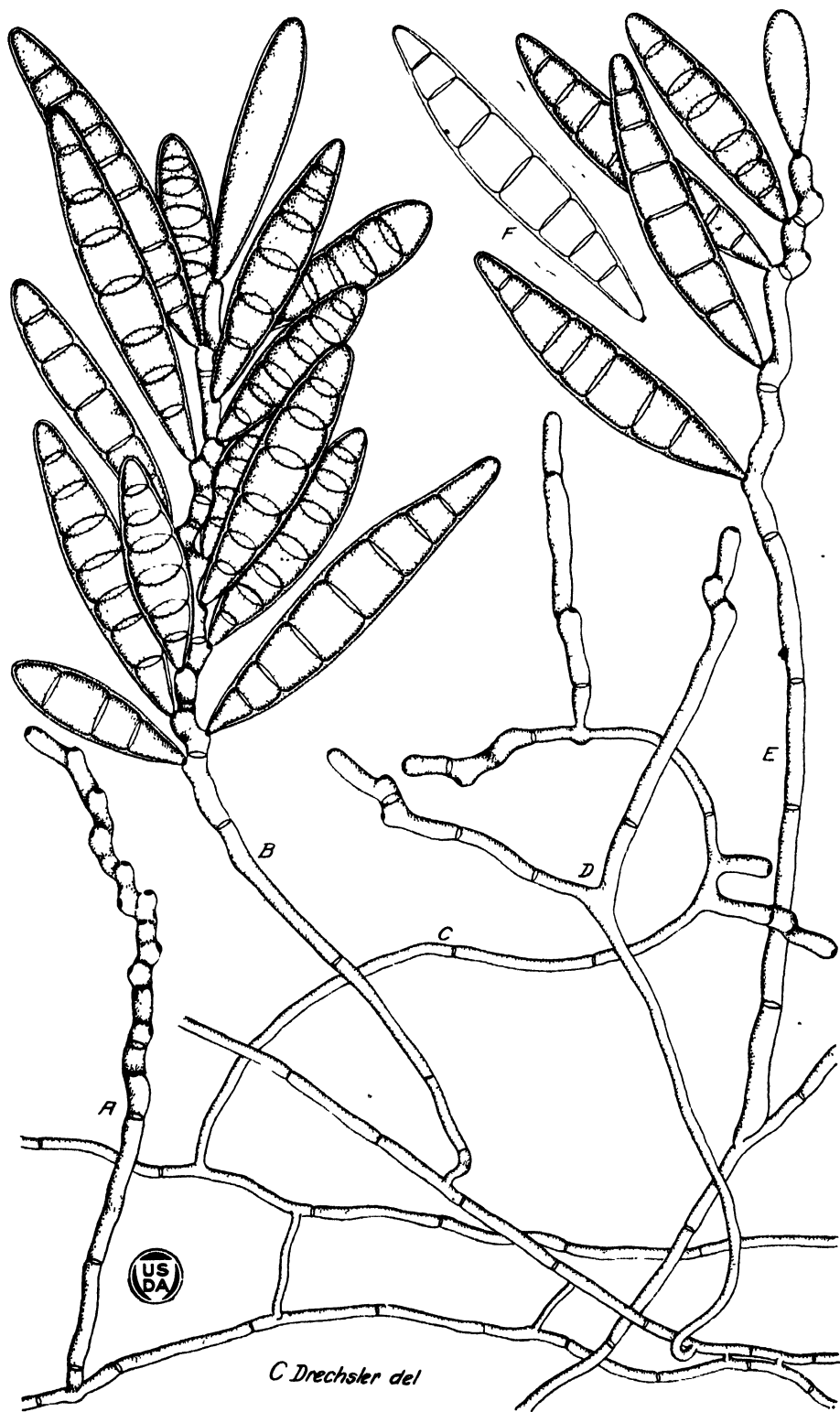
Ea-c -- Conidia from *Echinochloa crusgalli* germinating by the production of two polar germ tubes  $\times 500$

PLATE 21

*Helminthosporium monoceras*

A E.—Conidiophores of *H. monoceras* arising from imbedded anastomosing mycelium, showing habit of growth, irregular ramifications and production of conidia in racemose arrangement.  $\times 500$ . Drawn from 20-day old culture on tap-water agar.

F.—Outline of conidium from pure culture, showing attenuated regions in peripheral wall at apex and at basal end.  $\times 500$ .



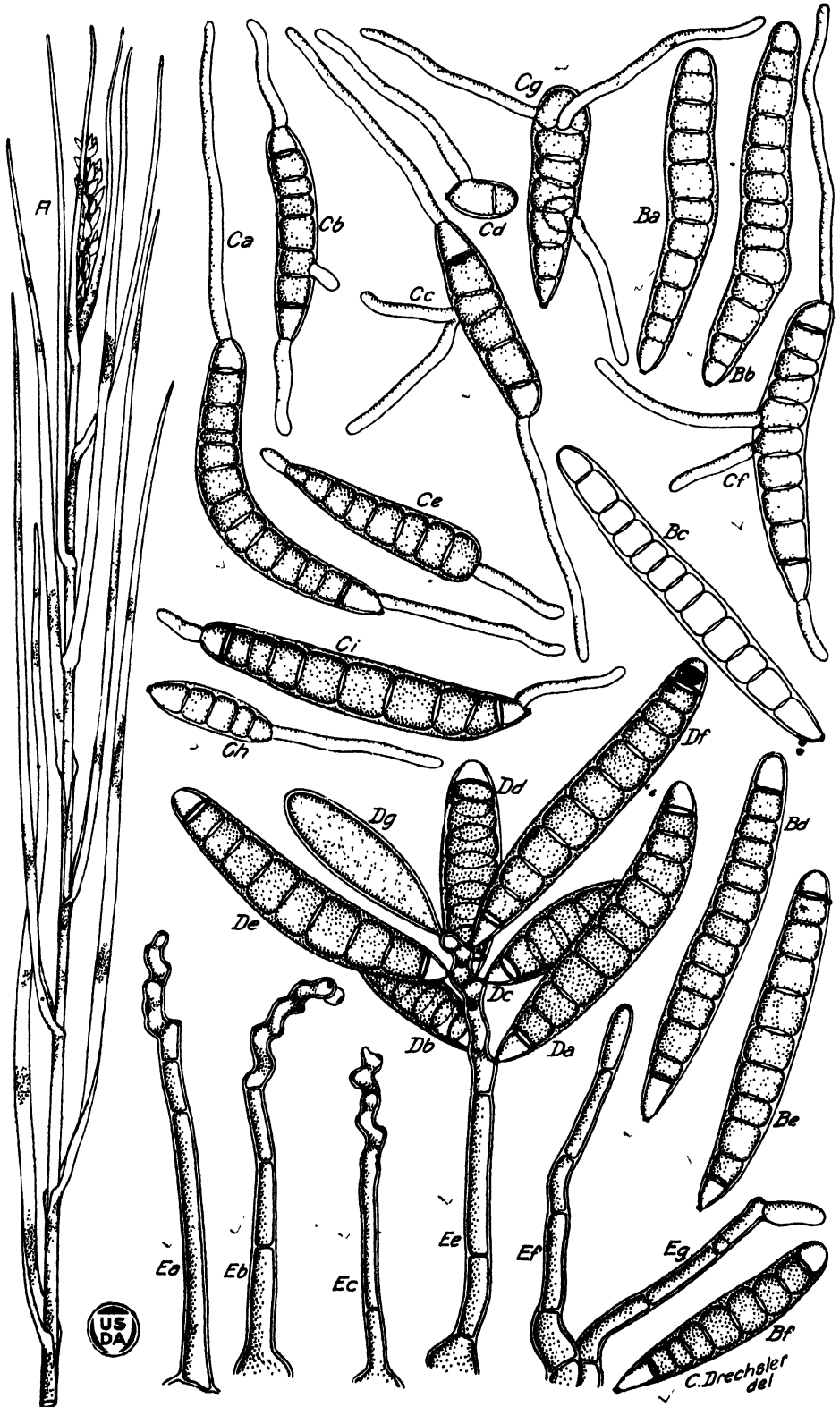


PLATE 22

*Helminthosporium halodes*

A.—Portion of *Distichlis spicata* showing discolored areas on blades and sheaths of leaves, due to attack by *H. halodes*  $\times 34$

Ba-f.—Conidia produced on leaf of *Distichlis spicata* in natural environment.  $\times 500$ .

Ca-i.—Conidia of *H. halodes* germinating in water. Ca-h, conidia produced on host in natural environment, Ci, conidium produced on portion of diseased leaf after incubation in damp chamber. Germination typical in Ca, c, h, i by production of two polar germ tubes, atypical in Cb, c, d, f, g by production of lateral germ tubes alone or in addition to polar germ tubes  $\times 500$

Da-f.—Conidia produced on diseased part of leaf of *Distichlis spicata* incubated in damp chamber  $\times 500$

Ea-g.—Conidiophores of *H. halodes*; Ea-e produced on host tissue incubated in damp chamber, Ef-g produced on host in natural environment  $\times 500$

PLATE 23

*Helminthosporium halodes*

A Conidiophore of *H. halodes* from tap-water agar culture, 30 days old, showing scars marking points of attachment of conidia relatively close together  $\times 500$ .

B.—Fructification of *H. halodes* developed on 30-day old water-agar culture, showing compact racemose arrangement of conidia.  $\times 500$

C. Conidiophore of *H. halodes* from 30 day old water-agar culture, showing branching and relationship to attached conidia  $\times 500$ .

D.—Irregularly curved spore developed on tap-water agar  $\times 500$

E.—Conidium produced on tap-water agar germinating typically by production of two polar germ tubes  $\times 500$ .

F.—Conidium produced on tap-water agar, showing bifurcating apex.  $\times 500$ .

G.—Conidium produced on tap-water agar, illustrating attenuation of peripheral wall at apex and immediately adjacent to hilum  $\times 500$ .

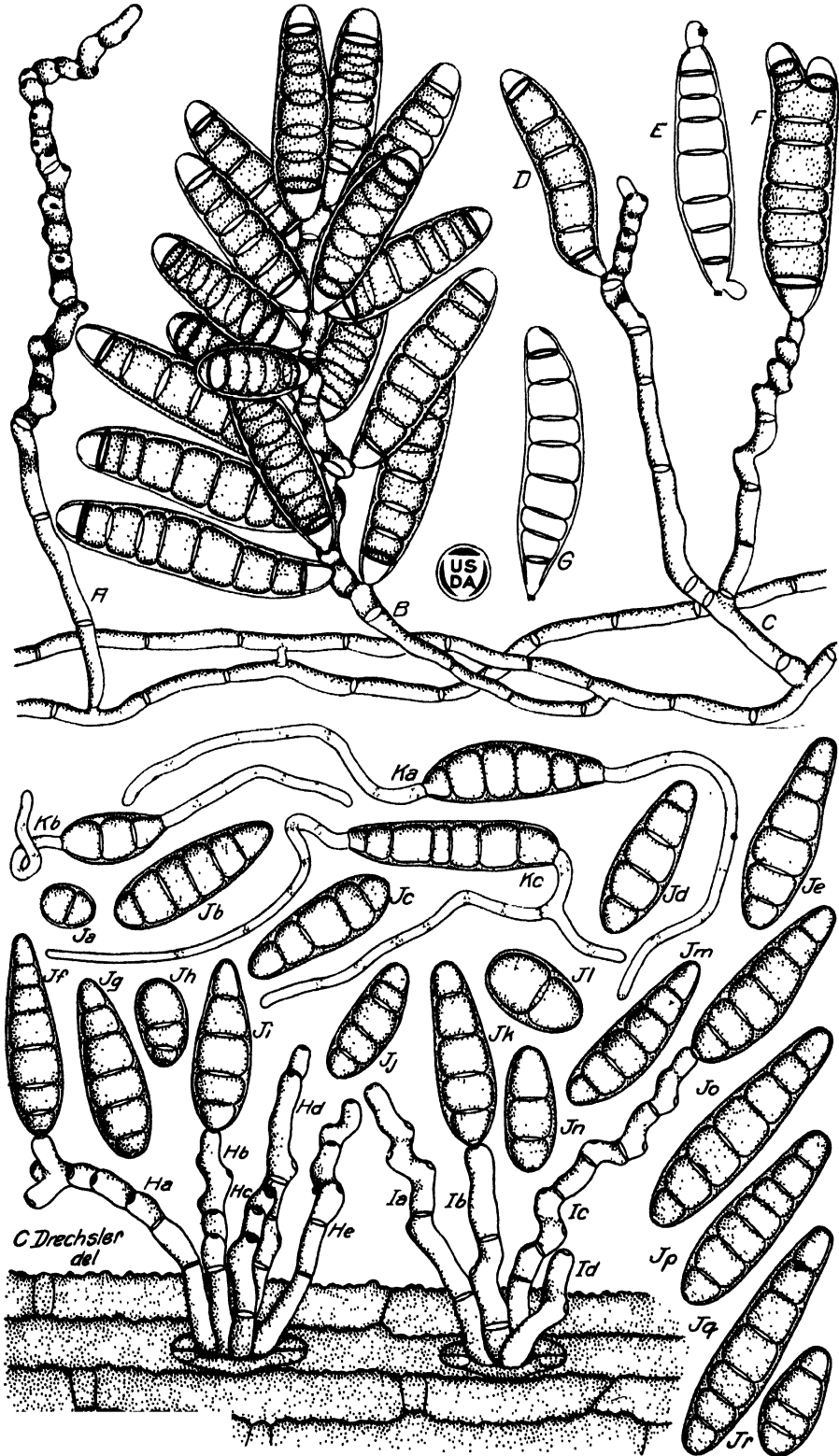
*Helminthosporium leucostylum*

Ha-c—Group of five conidiophores of *H. leucostylum* emerging from stoma of *Eleusine indica*.  $\times 500$ .

Ia-d. —Group of four conidiophores of *H. leucostylum* emerging from stoma of *Eleusine indica*.  $\times 500$

Ja-r.—Conidia from leaf of *Eleusine indica* showing variation in size, shape, and septation.  $\times 500$ .

Ka-c —Conidia from leaf of *Eleusine indica* showing typical germination by the production of two polar germ tubes.  $\times 500$ .





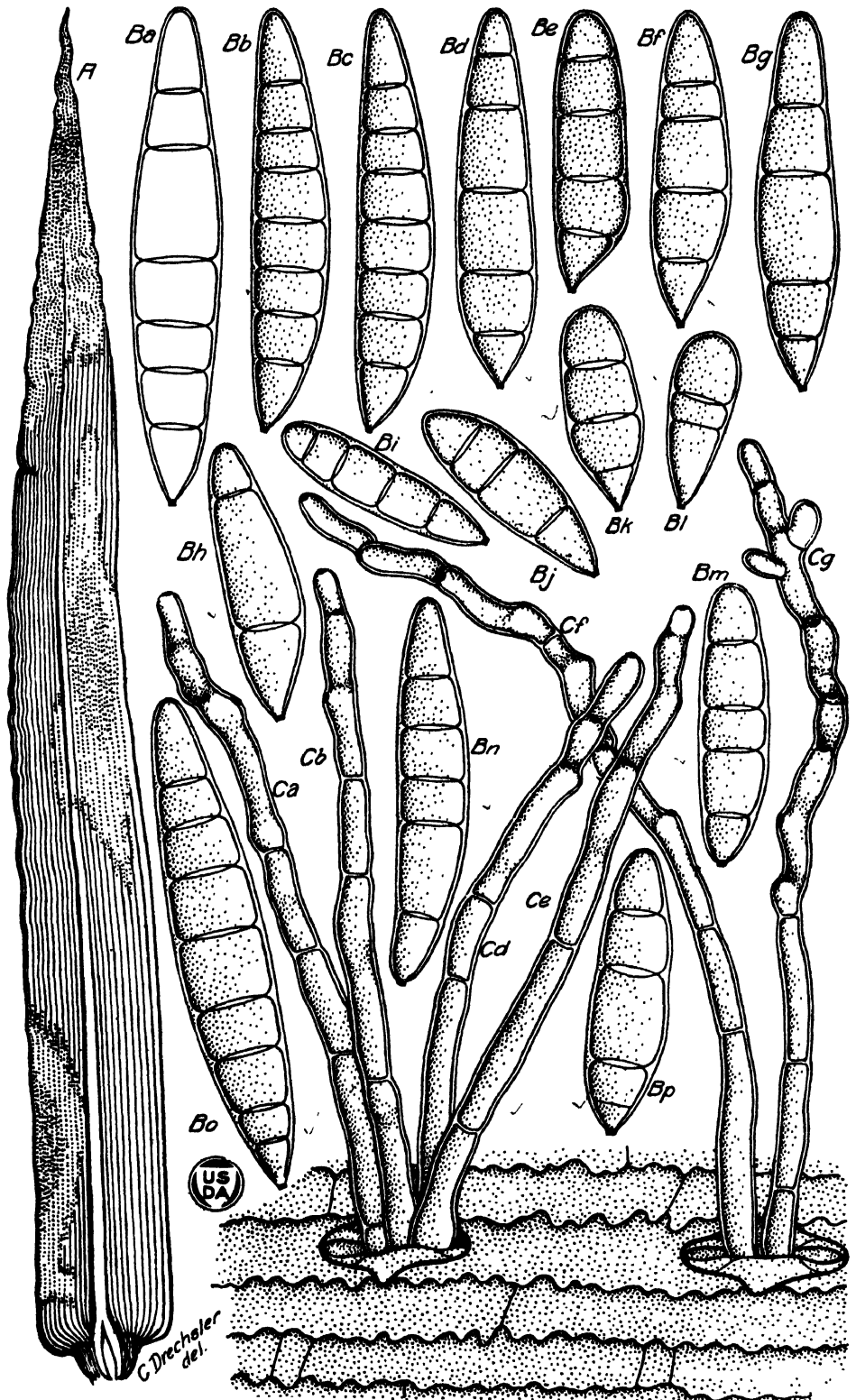


PLATE 24

*Helminthosporium turcicum*

A.—Leaf of sweet corn attacked by *H. turcicum*, showing extensive dry areas bearing fructifications near the center, and surrounded by slightly discolored margin.  $\times 13$ . Drawn from material collected near Valley Stream, N. Y., September 16, 1920

Ba-p.—Conidia of *H. turcicum* produced on leaves of sweet corn, showing variation in size, shape, and septation.  $\times 500$ .

Ca-g.—Conidiophores emerging in groups from stomata of sweet corn.  $\times 500$ .

PLATE 25

*Helminthosporium turcicum*

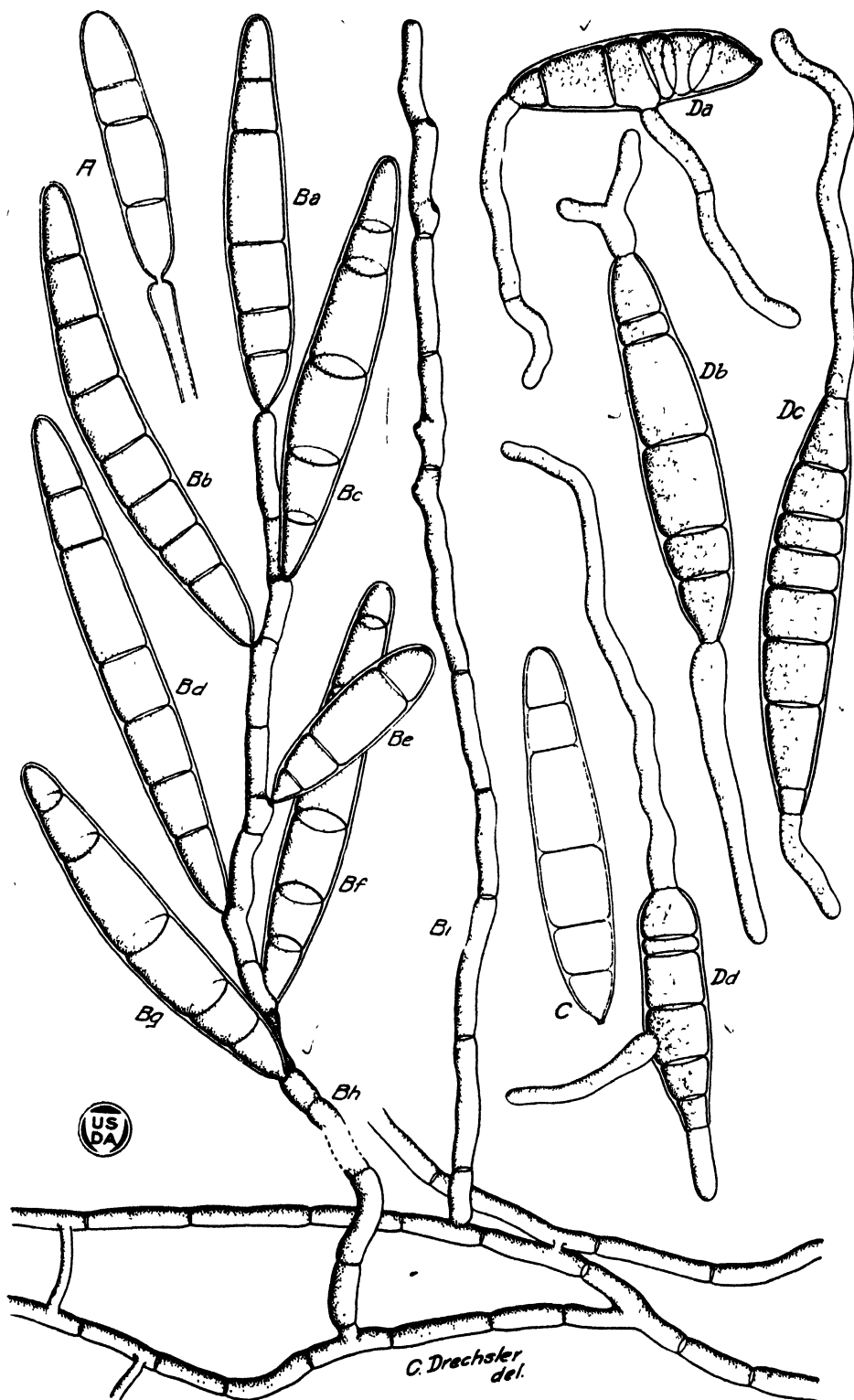
A.—Outline of conidium of *H. turcicum*, showing attachment to conidiophore.  $\times 500$ . From material grown in 20-day old tap-water agar culture.

Ba-Bg.—Conidia of *H. turcicum* developed in pure culture on tap-water agar  $\times 500$ .

Bh-i —Conidiophores from 20-day old tap-water agar culture, showing relation to mycelium.  $\times 500$ .

C.—Outline of conidium from diseased corn leaf, showing relation of hilum to basal segment.  $\times 500$ .

Da-d.—Conidia germinating in water, either typically (*Dh, c*) by the production of two polar germ tubes; or atypically (*Da, d*) by the proliferation of a lateral germ tube in addition to one or two polar tubes.  $\times 500$



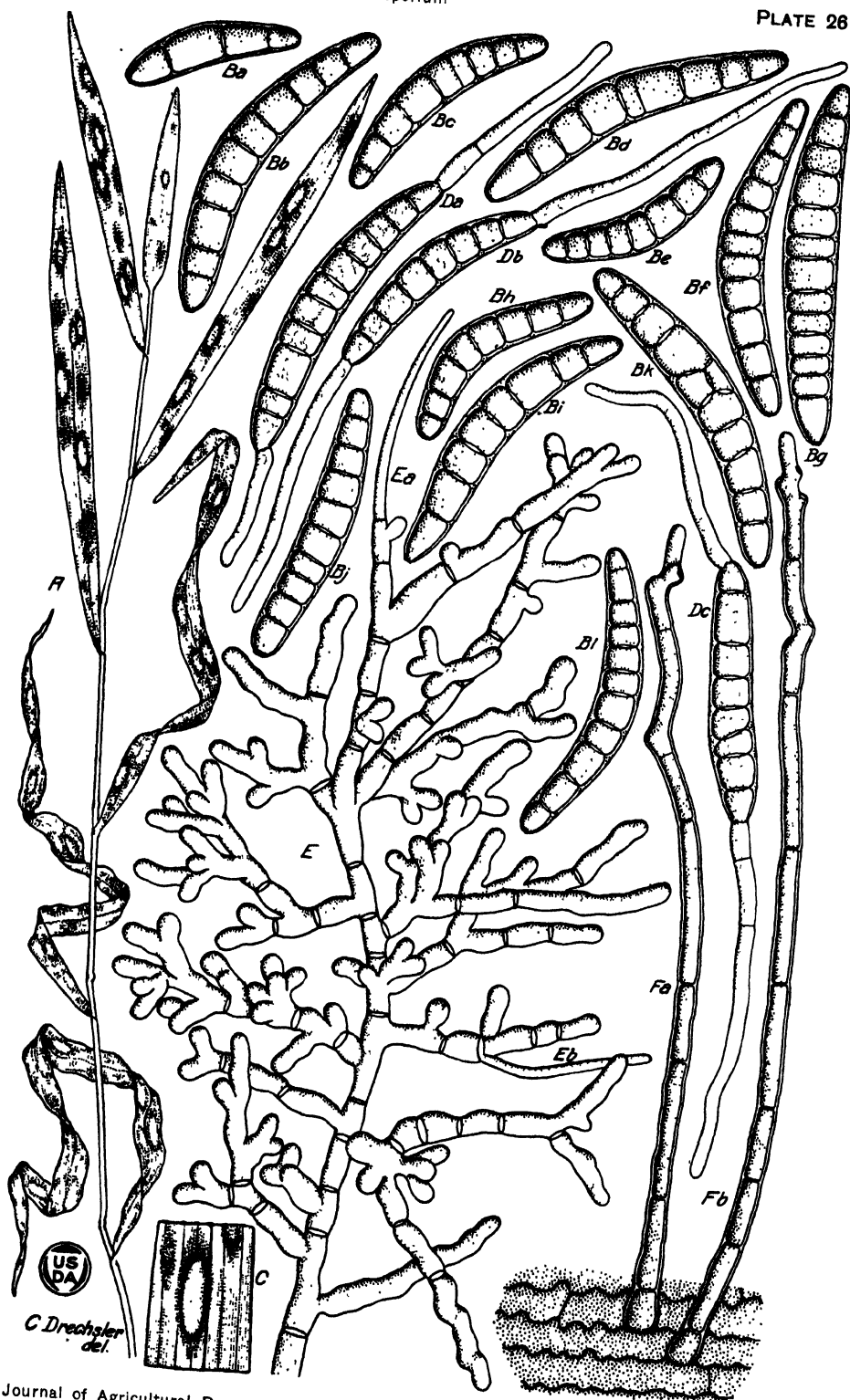


PLATE 26

*Helminthosporium leersii*

A.—Portion of plant of *Leersia virginica* attacked by *H. leersii* showing foliar spots in various stages of development and resultant withering of successively younger leaves.  $\times 1$ . Drawn from material collected near Meriden, Conn., September 7, 1920.

Ba 1. —Conidia of *H. leersii* produced on *Leersia virginica* under natural conditions, showing variations in size, shape, and septation.  $\times 500$

C — Portion of leaf of *Leersia virginica* attacked by *H. leersii*, showing foliar "eye spot" in detail  $\times 3$ .

Da-c —Conidia from diseased leaf of *Leersia virginica* germinating by the production of two polar germ tubes  $\times 500$

E — Ramifying system of short hyphal elements produced in 10-day old potato dextrose agar culture. Ea, b represent stolon-like elements, by the elongation of which the mycelium becomes more extensive.  $\times 500$

Fa-b. —Conidiophores of *H. leersii* produced on leaf of *Leersia virginica* under natural conditions.  $\times 500$ .

PLATE 27

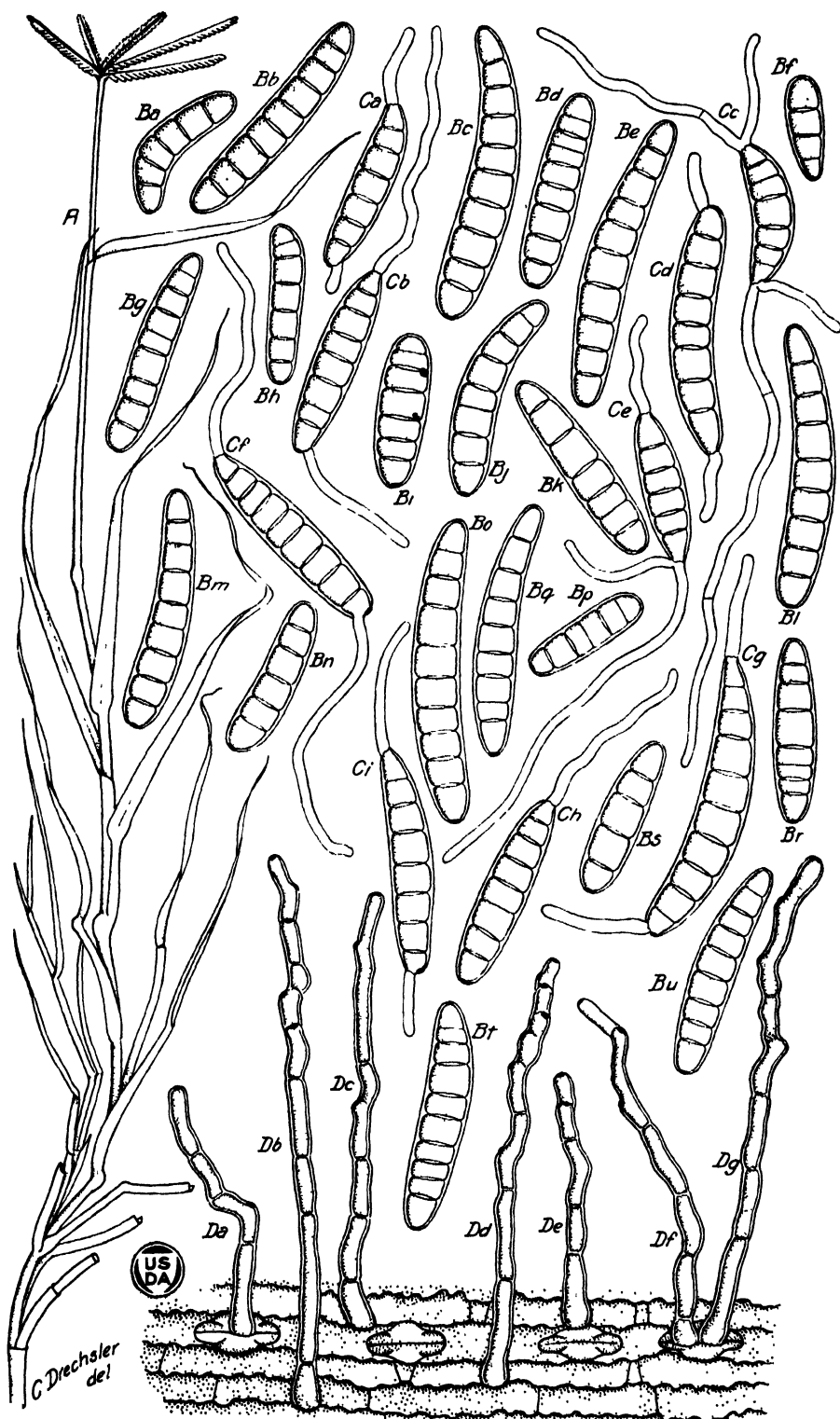
*Helminthosporium cynodontis*

A.—Portion of plant of *Cynodon dactylon* attacked by *Helminthosporium cynodontis*, showing withered condition of leaves bearing fructifications of fungus.  $\times 34$ . Drawn from material collected at Wauchula, Fla., April 19, 1921.

Ba-u.—Conidia from affected leaf of *Cynodon dactylon*, showing variation in size, shape, and septation.  $\times 500$ .

Ca-i.—Conidia from leaf of *Cynodon dactylon* germinating in water, by production of two polar germ tubes.  $\times 500$ .

Da-g.—Conidiophores of *H. cynodontis* emerging singly or in pairs from stomata or between epidermal cells of *Cynodon dactylon*.  $\times 500$ .





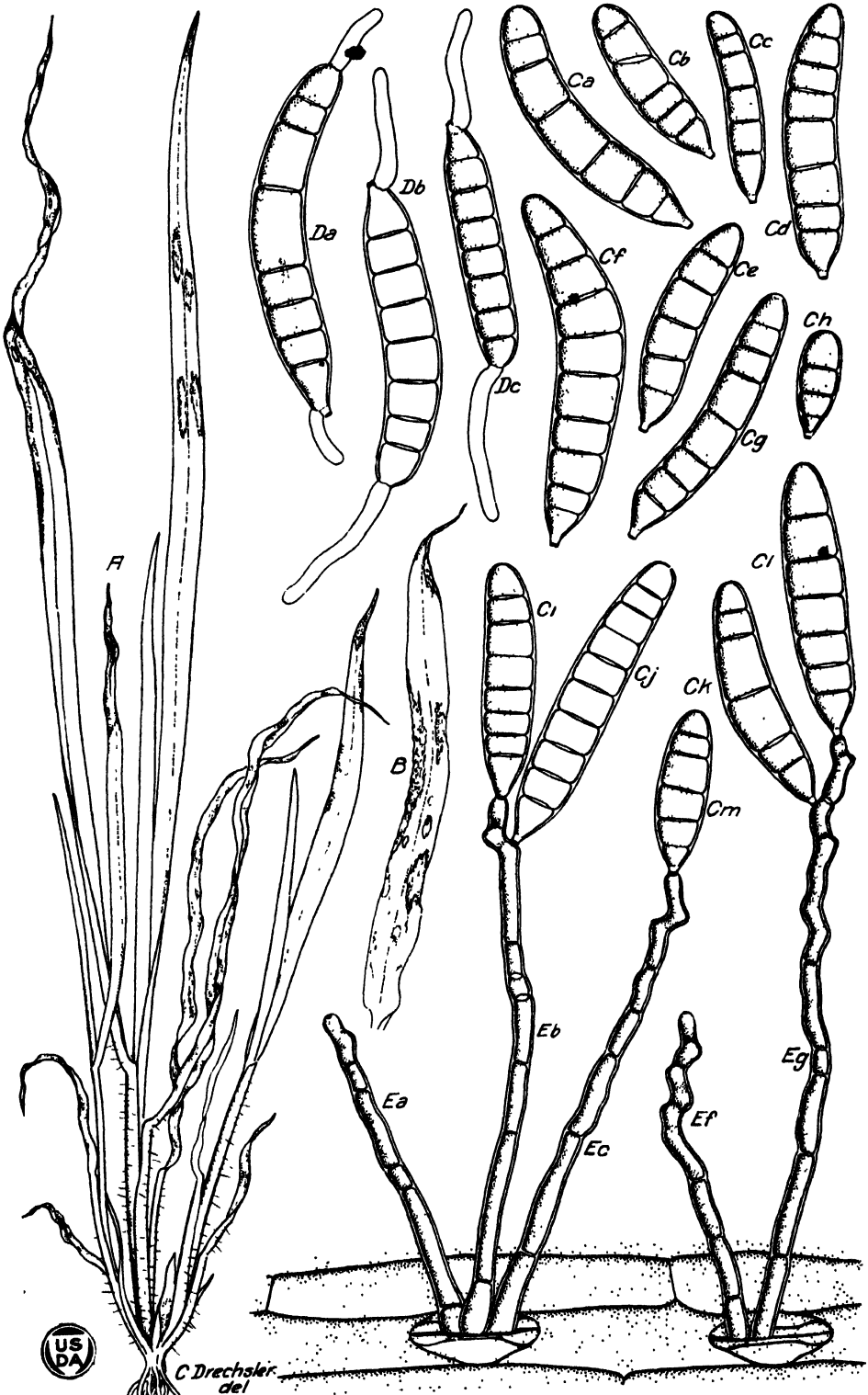


PLATE 28

*Helminthosporium micropus*

A.—Young plant of *Paspalum boscianum* attacked by *H. micropus*, showing distribution of affected regions and the withering of foliage caused by fungus.  $\times 34$ .

B.—Leaf of young plant of *Paspalum boscianum* attacked by *H. micropus*, showing several affected regions and distortion and withering of diseased foliar organ.  $\times 34$ .

Ca-m. Conidia from leaf of *Paspalum boscianum*, showing variation in size, shape, and septation.  $\times 500$ .

Da-c. —Conidia from leaf of *Paspalum boscianum* germinating in water by production of two polar germ tubes.  $\times 500$ .

Ea-g.—Conidiophores of *H. micropus* emerging in groups from stomata of *Paspalum boscianum*.  $\times 500$ .

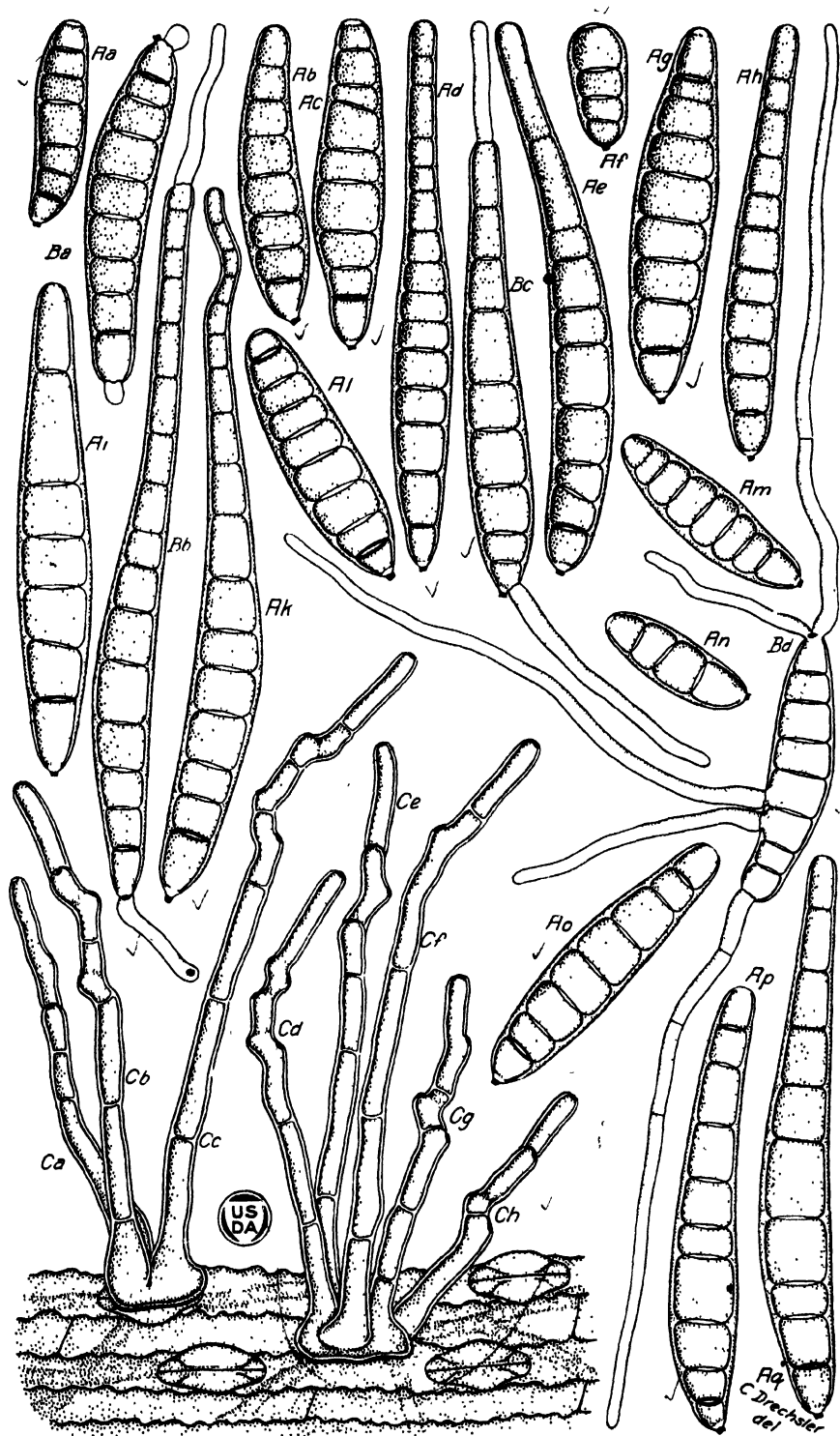
PLATE 29

*Helminthosporium rostratum*

Aa-q.—Conidia of *H. rostratum* from dry leaves of *Eragrostis major* collected near Washington, D. C., October 13, 1921, showing variation in size, shape, and septation.  $\times 500$ .

Ba-d.—Conidia from dry leaf of *Eragrostis major* germinating in tap water, the mature spores (*Ba-c*) by the production of two polar germ tubes; the newly proliferated spore (*Bd*) by the production of two lateral germ tubes in addition to polar tubes.  $\times 500$ .

Ca-h.—Conidiophores showing origin in groups from an expanded base and relation of latter to stomata or epidermal cells.  $\times 500$ .



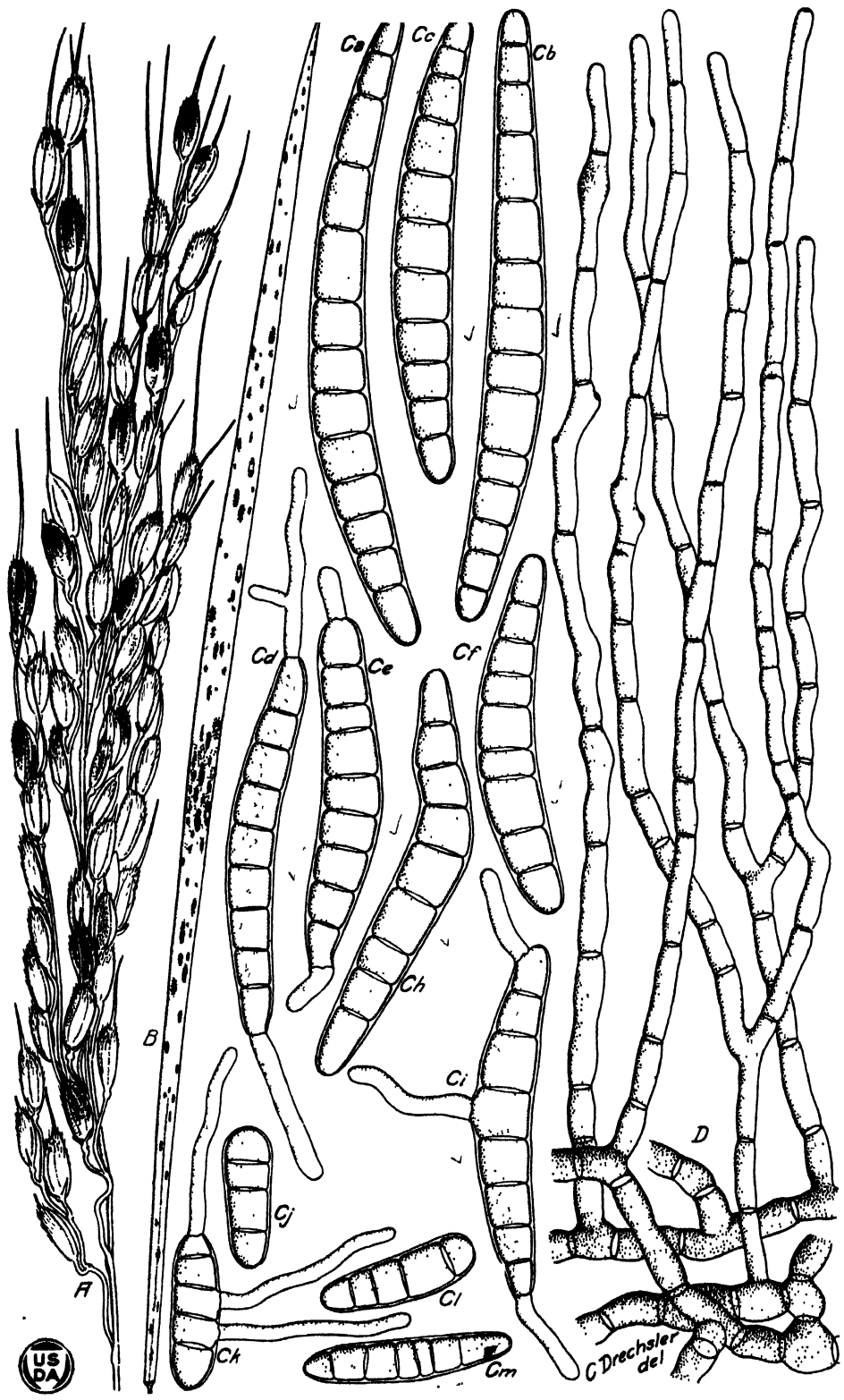


PLATE 30

*Helminthosporium oryzae*

A —Panicle of rice attacked by *H. oryzae*, showing some spikelets partly covered with dark growth composed of fructifications of the fungus.  $\times 1$ .

B —Leaf of rice showing numerous discolored spots resulting from inoculation with *H. oryzae*  $\times 3_4$ .

Ca-m —Conidia of *H. oryzae* produced on diseased floral parts. *Ca-b*, very large spores from well developed mats of fructification; *Cc, f, h, j, l, m*, conidia more typical in size from scattered fructifications; *Cd, e*, conidia germinating typically by the production of two polar germ tubes; *Cl, k*, relatively immature conidia germinating atypically by production of lateral germ tubes from intermediate segments in addition to polar germ tubes  $\times 500$ .

D —Conidiophores of *H. oryzae* from infected floral scale showing origin from stout prostrate hyphae.  $\times 500$ .

PLATE 31

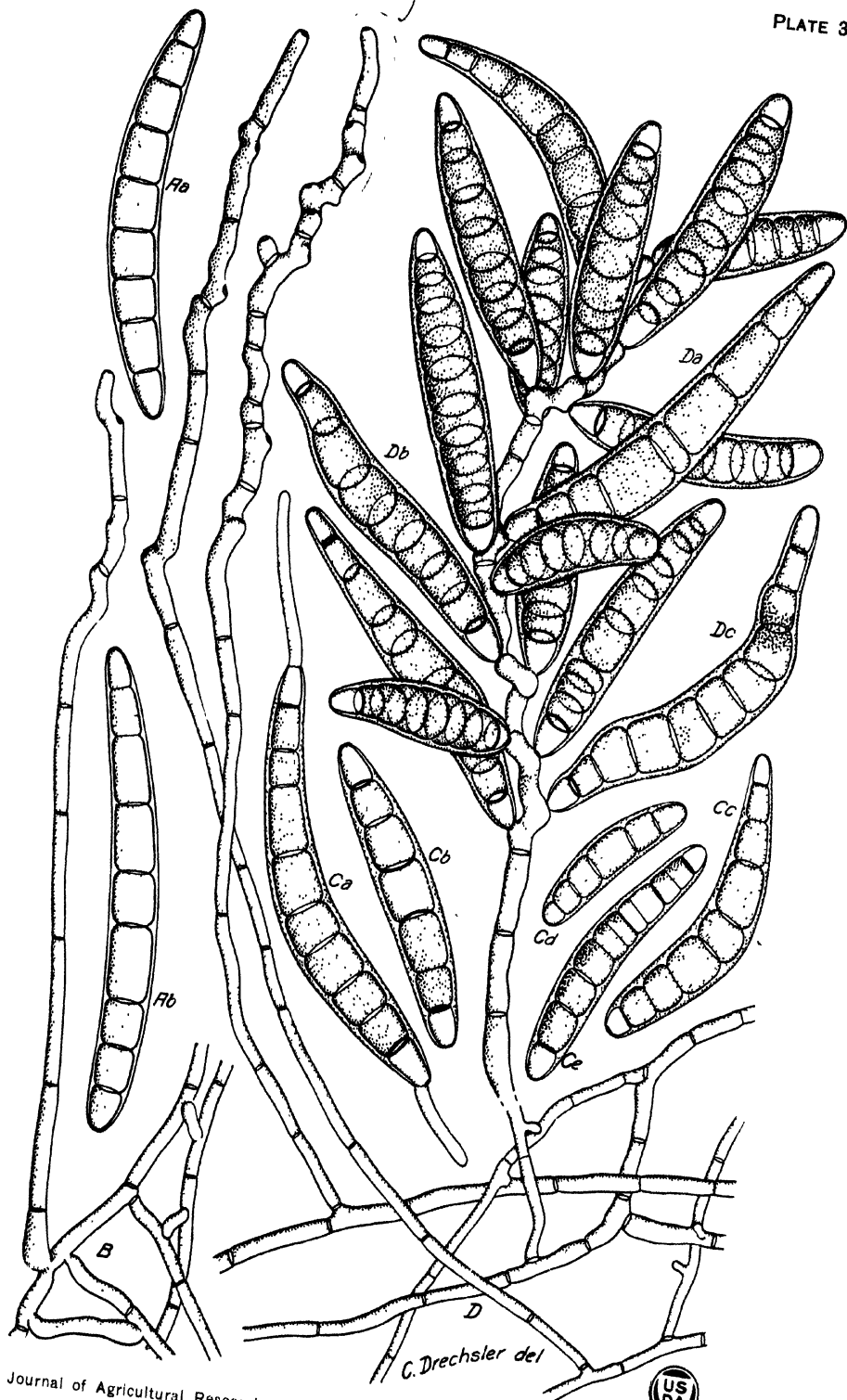
*Helminthosporium oryzae*

Aa-b. —Subhyaline conidia of *H. oryzae* from tap-water agar culture, 30 days old.  $\times 500$ .

B.—Conidiophore from tap-water agar culture, 30 days old, showing relation to vegetative mycelium.  $\times 500$ .

Ca-e.—Conidia from potato-dextrose agar culture, 30 days old; *Ca*, conidium germinating typically by production of two polar germ tubes; *Cb*, *c*, Conidia showing subhyaline end and intermediate segments, *Cc*, Conidium with two subhyaline end cells; *Cd*, Conidium of uniform color without subhyaline segments.  $\times 500$ .

D.—Fructification and denuded conidiophores developed on potato dextrose agar, 30 days after inoculation, showing relation of sporophores to vegetative hyphae, angle at which conidia are attached, and variation of the latter in shape, size, and septation.  $\times 500$ .





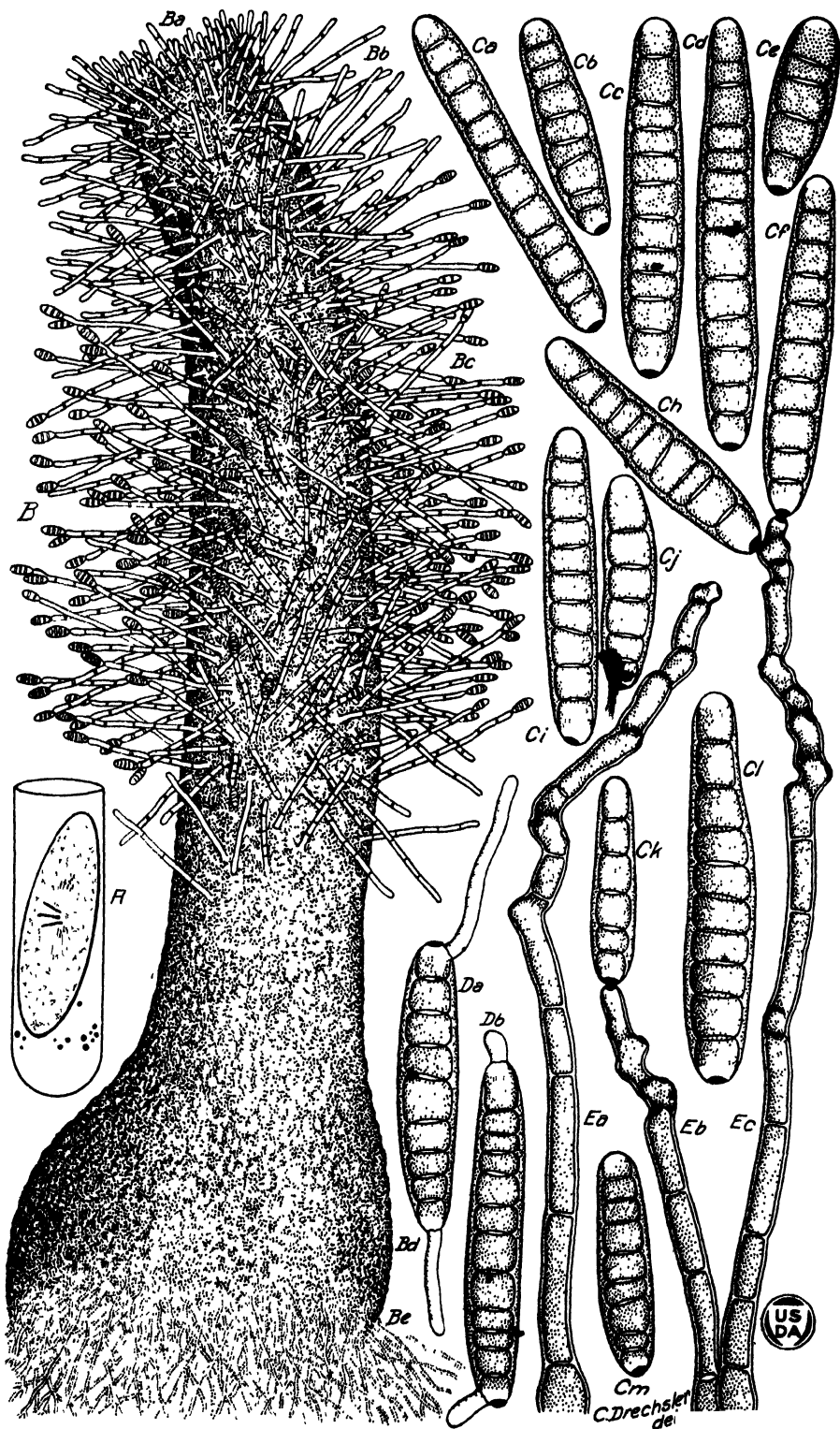


PLATE 32

*Helminthosporium cyclops*

A.—Culture of *H. cyclops* on Beijerinck's agar, 20 days after inoculation, showing subspherical sclerotia imbedded in substratum, discrete fructifications scattered sparingly over surface of substratum, fluffy mycelial growth near point where inoculum was planted, and three compound fructifications in center.  $\times 3/4$ .

B—Compound conidial fructification on Beijerinck's agar, showing origin from imbedded hyphae at base (*Bc*), sterile basal portion (*Bd*), conidiophore more or less radially arranged (*Bc*), and growing apex (*Ba*) where new conidiophores are proliferated (*Bb*).  $\times 500$

Ca-m.—Conidia from leaves of *Danthonia spicata*, showing thick peripheral wall, conspicuous hilum, and variation in size, shape, and septation.  $\times 500$ .

Da b --Conidia from leaves of *Danthonia spicata* germinating in water by the production of two polar germ tubes  $\times 500$

Ea c.—Conidiophores from leaf of *Danthonia spicata*.  $\times 500$ .

PLATE 33

*Helminthosporium cyclops*

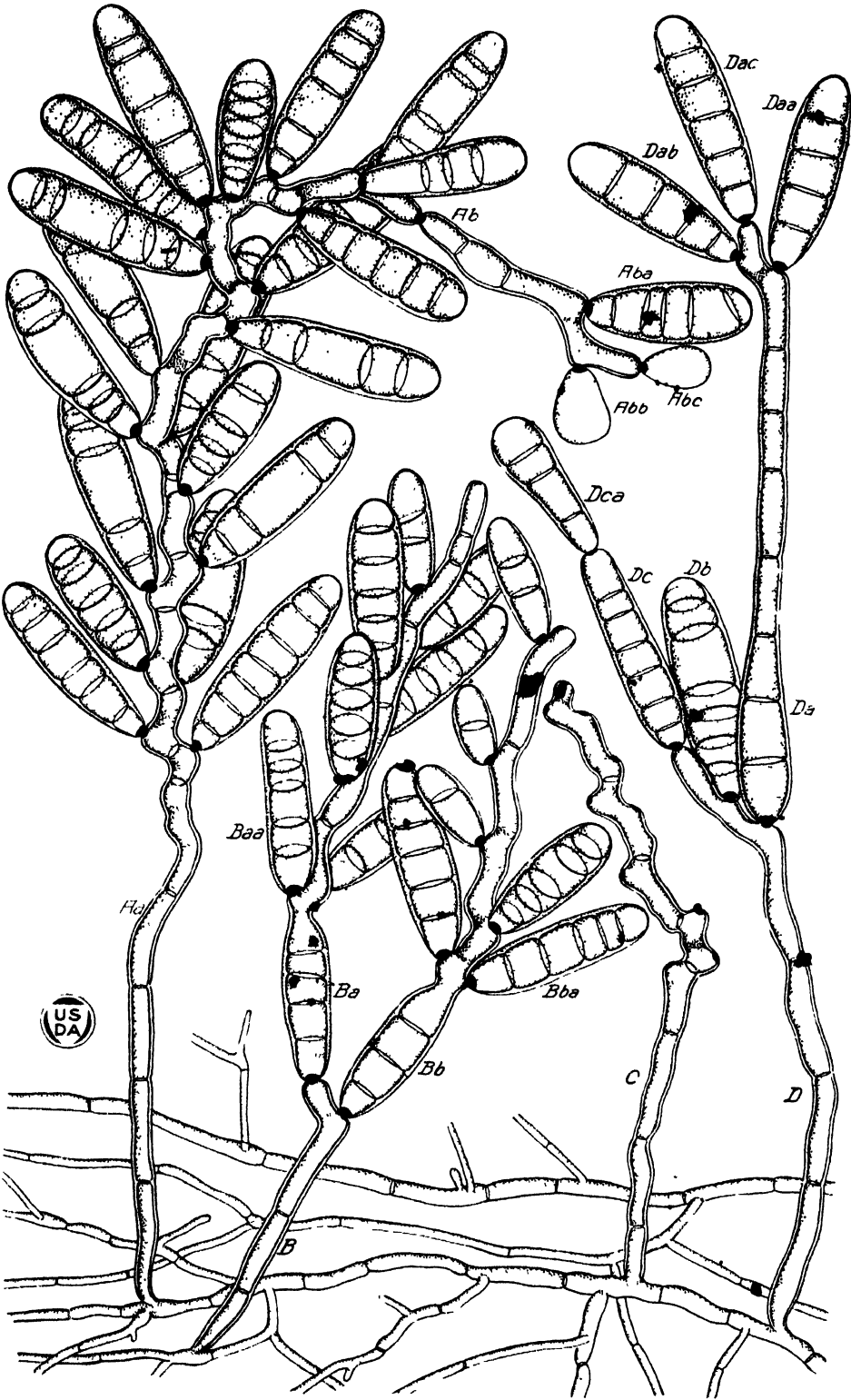
Mycelium, fructifications, and denuded sporophore of *H. cyclops* developed on Beijerinck's agar and drawn 30 days after inoculation of substratum.  $\times 500$ .

Aa.—Conidiophore which, after producing many spores, has proliferated the distal sporophoric element *Ab* by a budding process similar to the proliferation of a conidium. The distal sporophoric element has given rise to three spores, *Aba-bc*.

B.—Conidiophore, the typical development of which has been replaced after the proliferation of two primary conidia *Ba b*, by the growth of the latter into sporophoric elements, on which were developed numbers of secondary conidia, *Baa*, *Bba*, etc., respectively.

C.—Denuded conidiophore.

D.—Conidiophore bearing three primary conidia, one of which (*Da*) has grown out into a sporophoric process bearing three secondary spores *Daa-ac*; while another (*Dc*) has given rise directly to a secondary conidium *Dca* by apical proliferation.





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No. 9

## CONTROL OF SNOW MOLDING IN CONIFEROUS NURSERY STOCK<sup>1</sup>

By C. F. KORSTIAN<sup>2</sup>

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During the time that the Cottonwood Nursery of the United States Forest Service was in operation (1906-1921) very serious winter losses of Douglas fir (*Pseudotsuga taxifolia*) seedlings and transplants occurred under the snow. Several other species were also injured, the extent of loss varying with the species, age class, and time of snow disappearance in the spring. All age classes of Norway spruce (*Picea excelsa*) were especially susceptible, while Engelmann spruce (*Picea engelmanni*) and the Pacific coast form of western yellow pine (*Pinus ponderosa*) suffered only moderate injury, and lodgepole pine (*Pinus contorta*) was practically immune.

The Cottonwood Nursery is situated 25 miles southeast of Salt Lake City, Utah, on the Wasatch National Forest, at an elevation of 7,450 feet. Here the snow usually covers the ground continuously from November 1 to May 10, lasting occasionally even until May 20 or 25. The maximum depth varies from 6 to 8 feet. Under this heavy blanket of snow the ground seldom freezes, and even where freezing has occurred in the autumn before the advent of permanent snow, the soil soon thaws out after the snow begins to accumulate. Invariably the injury was greatest during years of heaviest snowfall and consequent late melting of snow in the spring.

Hartley, Pierce, and Hahn<sup>3</sup> studied this type of injury and found that it was caused by weakly parasitic fungi, attacking the leaves. They succeeded in isolating, by cultural methods, a number of organisms from recently snow-molded seedlings of Douglas fir, of which they most strongly suspected *Botrytis cinerea* and a dark sterile mold, as yet unidentified, as causing the disease. These investigators conducted spraying experiments with fungicides, including sulphuric acid, formaldehyde, zinc chloride, copper sulphate, copper acetate, and ammoniacal copper carbonate, none of which gave promise of effectively controlling the disease. A lime-sulphur mixture gave only very slightly beneficial results. Mulches consisting of thin layers of sawdust, sterilized sand, and gravel gave no promise. At the beginning of the present investigation

<sup>1</sup> Accepted for publication Sept. 4, 1922.

<sup>2</sup> The writer is indebted to former Forest Planting Assistant N. J. Fetherolf for painstaking assistance in carrying out the experiments here reported.

<sup>3</sup> HARTLEY, CHAS. F. PIERCE, and HAHN, GLEN C. MOLDING OF SNOW-SMOTHERED NURSERY STOCK. *In Forest Pathology*, v. 9, p. 209-231. 1922.

the writer submitted diseased specimens to Dr. Carl Hartley, who reported that the organisms were evidently the same as those with which he had worked.

For several years the practice of sowing black soil on the snow early in the spring to hasten its melting was followed by the nurserymen, but even with this practice the losses were still so heavy that the growing of Douglas fir on a large scale at the Cottonwood Nursery was discontinued in 1916.

TABLE I.—Percentage of injury by snow-molding fungi in the several species and age classes, spring of 1920

Species.	Age class.	Dead.	Alive but injured.
		Per cent.	Per cent.
<i>Picea engelmanni</i> .....	1-0	31.3	0
	2-0	27.7	0
	2-1	9.9	0
	3-2	8.1	0
<i>Picea excelsa</i> .....	2-0	77.0	0
	2-1	63.0	22.3
	3-2	59.3	0
<i>Pseudotsuga taxifolia</i> .....	1-0	62.9	0
	2-0	62.7	0
<i>Pinus ponderosa</i> .....	1-0	93.2	6.8
	2-0	52.2	44.7
	2-1	15.2	12.3
<i>Pinus ponderosa scopulorum</i> .....	1-0	16.8	2.5
	2-0	7.8	3.4
	2-1	3.5	15.8

The extent of the injury by these fungi among the different species and age classes is shown in Tables I and II, which are based upon counts of from several hundred to as many as 2,400 seedlings in each age class. It is recognized that a small loss occurred during the summer seasons but it was found to be negligible in comparison with the loss due to snow molding. Table I and Plate 1 show the much greater susceptibility of the exotic Norway spruce to the disease as compared with that of the native Engelmann spruce. Table I shows quite clearly that the younger age classes, 1-0 and 2-0, suffer by far the greatest loss and injury. Table II fails to bring out this relationship so clearly mainly because of the fact that a large part of the older age classes of stock had been abandoned, receiving no water or care of any kind during the summer of 1920. This left the stock in a weakened condition and an easy prey for the fungi. For example, 3-3 Engelmann spruce shows (not in Table II) 41.5 per cent of dead and injured trees as compared with 3.4 per cent for the 3-2 stock and 7.1 per cent for the 3-1 stock. The first age class had been abandoned in 1920, while the latter two classes of stock were watered and otherwise properly cared for during 1920. It should be noted, therefore, that among the older age classes, especially transplants, it is usually the weak or previously injured trees that are killed or severely injured. It is not uncommon to find some of the leaves of vigorous transplants infected when the snow melts, but the development of the fungi is checked as soon as the trees are exposed to the sunlight, and complete recovery may follow. Jack pine (*Pinus banksiana*), however,

was found to be a notable exception. During the winter of 1919-1920 a bed of some 300 vigorous 4-year-old jack pine transplants, 18 inches tall, was completely killed by the disease. Tables I and II clearly show that the Pacific Coast form of western yellow pine (*Pinus ponderosa*), grown from seed collected in central Idaho, is much more susceptible to the snow-molding fungi than the Rocky Mountain variety of the same species (*Pinus ponderosa scopulorum*), grown from seed collected in the region near the nursery.

TABLE II.—Percentage of injury by snow-molding fungi in the several species and age classes, spring of 1921

Species.	Age class.	Dead.	Alive but injured.
		Per cent.	Per cent.
<i>Picea engelmanni</i> .....	3-0	8.4	13.3
	3-1	0.8	6.3
	3-2	0	3.4
<i>Picea excelsa</i> .....	3-0	80.4	11.7
	3-3	80.3	19.6
<i>Picea parryana</i> .....	3-0	60.2	30.8
	3-3	3.0	31.5
<i>Pseudotsuga taxifolia</i> .....	1-0	5.1	6.9
	2-0	16.2	19.3
	<sup>a</sup> 3-0	2.0	5.4
<i>Pinus ponderosa</i> .....	<sup>b</sup> 3-0	31.1	30.4
	1-0	11.5	3.5
	3-0	0	83.0
	2-1	37.2	38.0
	2-2	36.8	35.6
<i>Pinus ponderosa scopulorum</i> .....	1-0	0.8	1.6
	3-0	4.5	18.5
	2-1	23.7	26.8
<i>Pinus contorta</i> .....	2-2	6.3	24.7
	2-1	0	3.0
<i>Abies concolor</i> .....	2-2	0	1.5
	3-0	22.1	30.8
<i>Pinus resinosa</i> .....	2-1	0	15.8
<i>Pinus strobus</i> .....	2-1	60.2	39.8
<i>Libocedrus decurrens</i> .....		4.1	8.4
<i>Thuja occidentalis</i> .....		100.0	0
<i>Juniperus monosperma</i> .....		100.0	0

<sup>a</sup> Bed protected by framework of 2 by 6 lumber.

<sup>b</sup> Unprotected bed.

Since most fungi will gain a foothold more readily at some previously injured part of a plant, it seems quite probable that the fungous injury during the winter of 1919-1920 was greatly increased as a result of the severe injury to the ends of the twigs by frost in the spring of 1919.<sup>4</sup>

In the spring of 1918 the problem of working out an efficacious method of controlling the snow molding of coniferous nursery stock was taken up by the writer. Six 4 by 12 foot beds were sown to the same amounts of Douglas fir, the time and method of sowing and subsequent care throughout the summer season being the same. Uniformly good stands were secured on all of the beds. The seed beds were covered with shade frames giving half shade during the hot, dry portion of the summer. In the autumn the shade frames were removed and just before the

<sup>4</sup> KORSTIAN, Clarence F. EFFECT OF A LATE SPRING FROST UPON FOREST VEGETATION IN THE WASATCH MOUNTAINS OF UTAH. In Ecology, v. 2, p. 47-50, 1 fig. 1921. Literature cited, p. 52.



permanent winter snow came, different treatments were applied to five of the beds, while the sixth was left untreated as a control. The object of these special treatments was mainly to afford mechanical protection from the disease. The treatment which gave much the best results consisted of a framework of 2 by 6 inch planks loosely placed with a spacing of 1 to 2 inches upon logs or squared timbers lying lengthwise along the sides of the beds. This type of protective covering, which was used on Bed 14, is shown in Plate 2, B. Two-inch lumber was used, mainly because it was available. It is possible that one-inch lumber could be used, if it were adequately supported to hold the heavy mass of snow above the tops of the seedlings. As soon as the snow had melted sufficiently in the spring to permit, the framework was removed. It was placed on the bed each autumn before the advent of the heavy winter snowfall.

TABLE III.—*Survival of Douglas fir seedlings in beds receiving different treatments as indicated; sowing spring of 1918.*

Bed No.	Date of count.	Total germination.	Survival.		Special treatment.
			Total.	In per cent of total germination.	
		<i>Per square foot.</i>	<i>Per square foot.</i>	<i>Per cent.</i>	
12	Oct. 9, 1918	126	103	81. 8	None; control.
	Sept. 29, 1919		81	64. 2	
	July 1, 1920		45	35. 7	
	Oct. 6, 1920		42	33. 3	
	May 22, 1921		14	11. 1	
13	Oct. 9, 1918	163	131	80. 3	Mulched with timothy hay winters of 1918-19 and 1919-20, 1920-21.
	Sept. 29, 1919		24	14. 7	
	July 1, 1920		0	0	
	Oct. 6, 1920		0	0	
	May 22, 1921		0	0	
14	Oct. 9, 1918	128	98	76. 6	Framework of 2 by 6's, 2 inches apart supported by timbers several inches above tops of trees. Winters 1918-19, 1919-20, 1920-21.
	Sept. 29, 1919		95	74. 2	
	July 1, 1920		84	60. 0	
	Oct. 6, 1920		84	60. 0	
	May 22, 1921		84	60. 0	
15	Oct. 9, 1918	69	58	84. 0	Mulched with aspen leaves. Winters 1918-19, 1919-20, 1920-21.
	Sept. 29, 1919		24	34. 8	
	July 1, 1920		13	18. 8	
	Oct. 6, 1920		13	18. 8	
	May 22, 1921		13	18. 8	
16	Oct. 9, 1918	79	58	73. 4	Covered with canvas throughout the winters of 1918-19, 1919-20, 1920-21.
	Sept. 29, 1919		28	35. 4	
	July 1, 1920		3	3. 8	
	Oct. 6, 1920		3	3. 8	
	May 22, 1921		1	1. 3	
17	Oct. 9, 1918	134	96	71. 6	Mulched with sterilized sand. Winters of 1918-19, 1919-20, 1920-21.
	Sept. 29, 1919		78	58. 2	
	July 1, 1920		8	6. 0	
	Oct. 6, 1920		8	6. 0	
	May 22, 1921		5	3. 7	
7	Oct. 6, 1920	165	<sup>a</sup> 110	66. 6	Framework, 2 by 6's. Same as bed No. 14.
	May 22, 1921		<sup>a</sup> 100	60. 6	
9	Oct. 6, 1920	142	<sup>a</sup> 93	65. 4	None; control.
	May 22, 1921		<sup>a</sup> 23	16. 2	

<sup>a</sup> Sowing spring of 1920.

Since this investigation was completed Cornefert<sup>6</sup> has reported a somewhat different method which has given excellent results for several years in the protection of spruce nursery stock from *Herpotrichia nigra*. In the autumn peeled poles 10 to 15 cm. in diameter are placed between the rows of plants, so that the plants lean upon them when pressed down by the snow. In field planting each tree is placed so that it can be supported by a rock or a stump.

Table III shows the original germination per square foot, survival on various dates, and the special treatment applied to each of the beds sown in 1918. The use of the protective framework of 2 by 6 inch planks was again checked during the winter of 1920-21 on Douglas fir seedlings originating from seed sown in the spring of 1920, the survival data for which is also included in Table III. The general appearance of the 3-0 beds of the 1918 sowing are shown in Plate 2, A. From Table III it will be seen that the results are decidedly in favor of the plank framework (Bed 14 of the 1918 sowing and Bed 7 of the 1920 sowing), which keeps the heavy mass of snow from mashing the seedlings down flat against the soil. Sufficient snow sifts through the intervening spaces between the 2 by 6's to keep the soil moist during the winter. At the end of the third winter Bed 14 of the 1918 sowing showed an average survival of 84 seedlings per square foot, or 60 per cent of the original germination compared with the next highest survival of 14 seedlings per square foot, or 11.1 per cent of the original germination in Bed 12—the control bed to which no special treatment had been applied. During the winters of 1918-19 and 1919-20 the protected bed (No. 14) suffered losses of 2.4 per cent and 14.2 per cent and none at all during the succeeding winter, as compared with losses in the control bed (No. 12) during the three winters of 17.6, 28.5, and 22.2 per cent, respectively. Bed 7 of the 1920 sowing, which was given protection by the same type of framework covering as that described above, suffered a loss of 6 per cent during the winter of 1920-21, as compared with a loss of 49.2 per cent in the control bed during the same winter.

It is evident from Table III and from general observations in the nursery that a mulch of organic matter stimulates the activities of the fungi by providing a suitable substratum on which they may develop. Contact with various kinds of organic matter, dead snow-molded plants, and a surface even with the soil predisposes to the disease.

On May 25, 1921, the stock in Bed 14 (protected by framework) and in Bed 12 (control) was dug and graded with the result that 67 per cent of the stock from Bed 14 was suitable for transplanting, while only 34.4 per cent of the seedlings from Bed 12 was suitable. Even many of the firsts in the unprotected bed were infected with the snow-molding fungi, which probably would result in a heavier culling the following spring when the transplants were dug preparatory to field planting. The foliage was of a dull green color and numerous leaves were dead or partially killed. This lot of stock had very few healthy buds, only 25.5 per cent, while the seedlings from the protected bed had 90.7 per cent of good terminal buds. In each case the bud scales of the unhealthy seedlings were loose and flaccid, giving the buds a withered appearance. Small bunches of each lot of stock were allowed to stand in the laboratory with the roots submerged in water for 10 days. Practically all of the buds on the seedlings

<sup>6</sup> CORNEFERT, R. RÉGÉNÉRATION SUR LES HAUTS PLATEAUX DU JURA. *As Soc. Forest. Franche-Comté et Belfort* Bul. Trimest., t. 14, p. 206. 1921.

from the protected bed developed into normal growing shoots, while those from the unprotected bed remained in a dormant condition, apparently due to injury by these fungi.

Table IV and Plate 3 show the root, top, and terminal bud development of Douglas fir seedlings grown three years in protected and unprotected seed beds. The trees from the unprotected bed had heavier roots and tops and a greater stem diameter. This is probably due to the fact that the stand of seedlings in the unprotected bed is much more open than in the protected bed, and consequently each plant has more growing space in which to develop. At the same time none but the largest and most vigorous seedlings survived in the unprotected bed. On the other hand, the roots from the unprotected bed were heavier in proportion to the weight of the plant, the tops being correspondingly less. This is probably attributable to the fungous injury to the tops, which was unfavorable to their development and which was much less pronounced in the protected beds.

TABLE IV.—Root, top, and terminal bud development

Bed.	Average length of tops.	Average length of roots.	Average weight of tops.	Average weight of roots.	Ratio of weight of root to weight of entire plant.	Average diameter of stem at root collar.	Average length of terminal bud.	Average width of terminal bud.
	Inches.	Inches.	Gm.	Gm.	Per cent.	Inches.	Inches.	Inches.
Protected seedlings (Bed 14).....	6.03	11.43	1.62	0.46	22.1	0.08	0.24	0.11
Unprotected seedlings (Bed 12).....	5.77	10.68	1.78	.75	29.6	.10	.18	.09

The examination of field plantations revealed considerable fungous injury during the winter of 1919-1920, especially among jack pine, Engelmann spruce and Norway spruce. The jack pine were practically all dead and the few living trees so severely injured that ultimate recovery was impossible. With Norway spruce and Engelmann spruce, the greatest injury from these fungi occurred on soils rich in organic matter, the surface of which was covered with considerable plant litter. The underplanting of aspen (*Populus tremuloides*) stands with Engelmann spruce, Norway spruce, and Douglas fir has been accompanied by notable losses on areas on which the ground was covered with a deep layer of aspen leaves. The heaviest losses occur where the plants are bent over into intimate contact with the soil and litter which is the breeding ground of these weakly parasitic snow-molding fungi. Serious loss by molding under the snow can be prevented in spring plantings by removing the accumulation of leaf litter from an area of one or two square feet, in the center of which the hole for the tree is dug and also by the use of large vigorous planting stock, which will stand erect during leaf fall and through the wet snows of the early winter and in this way hold its leader out of the zone of greatest danger.

### CONCLUSIONS

The snow-molding fungi have caused a greater loss of coniferous nursery stock at the Cottonwood Nursery than any other form of injury or disease.

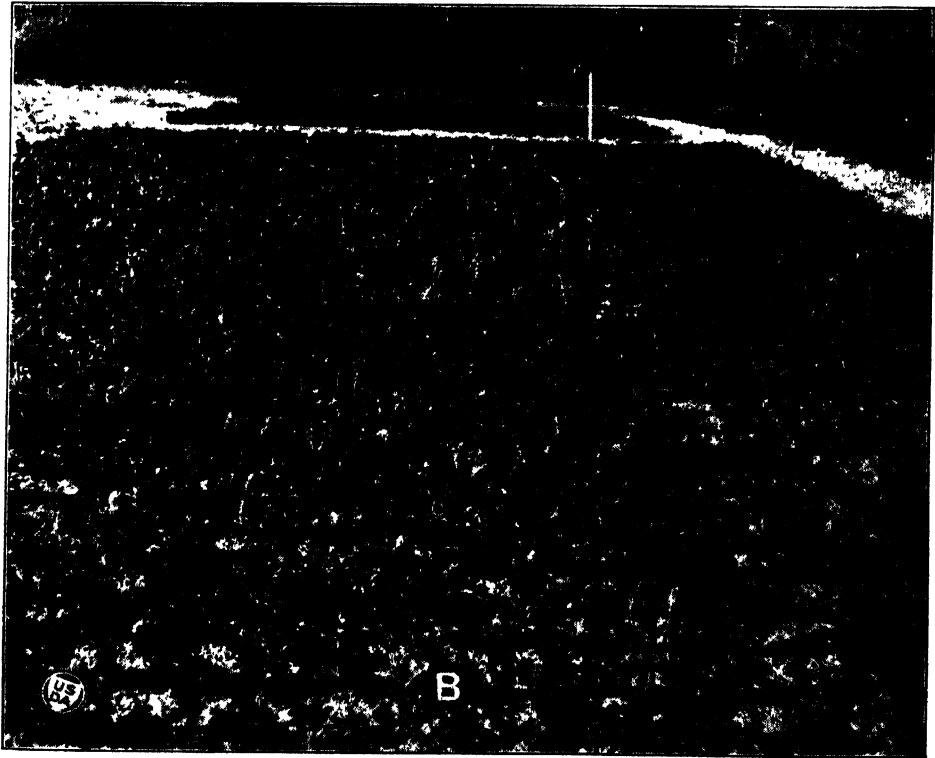
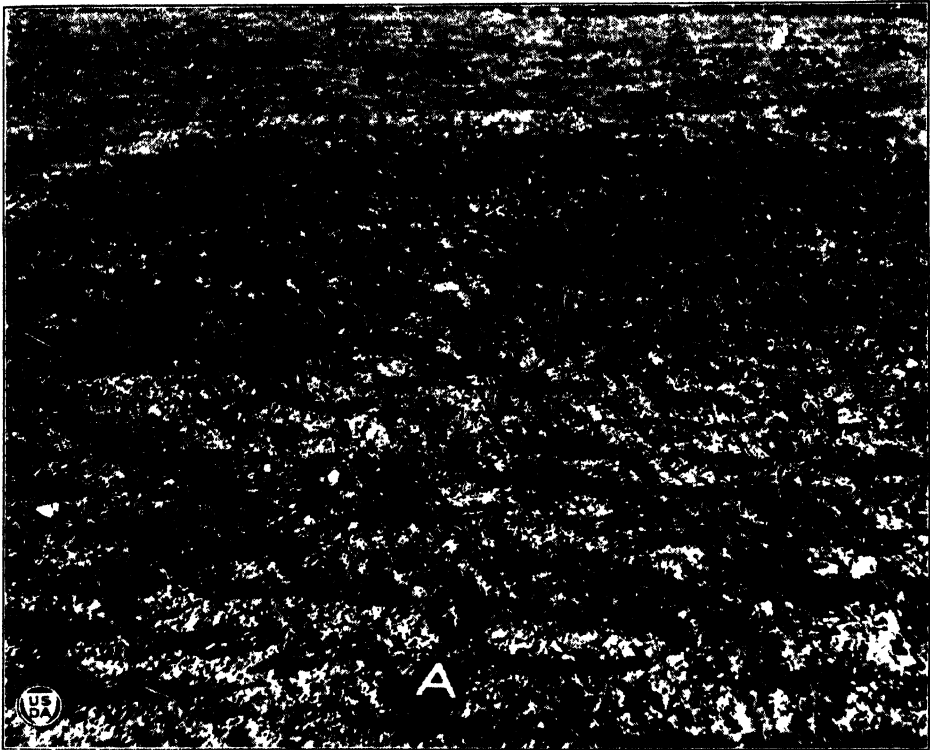
Examinations showed that practically all age classes and species grown in this nursery, excepting lodgepole pine, are subject to this disease and that it is more prevalent among young seedlings (1-0 and 2-0) than in the older age classes. Among older seedlings and transplants, the weak and previously injured trees are the ones generally attacked.

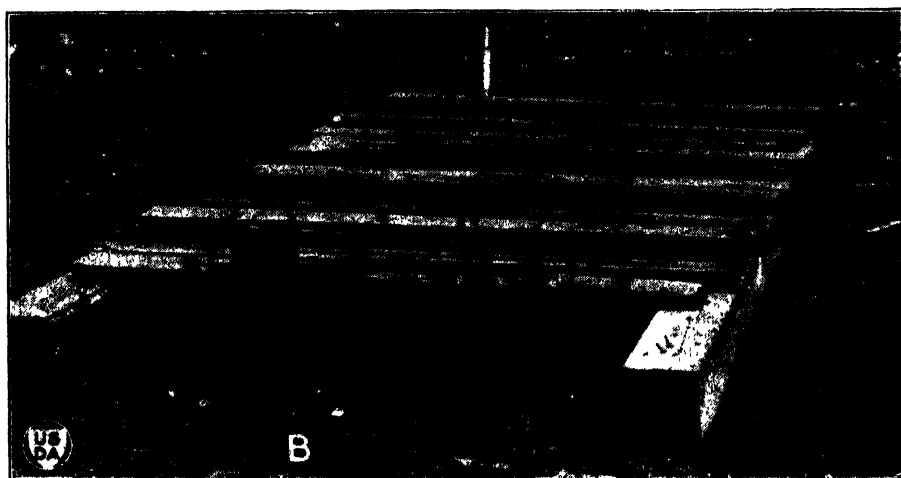
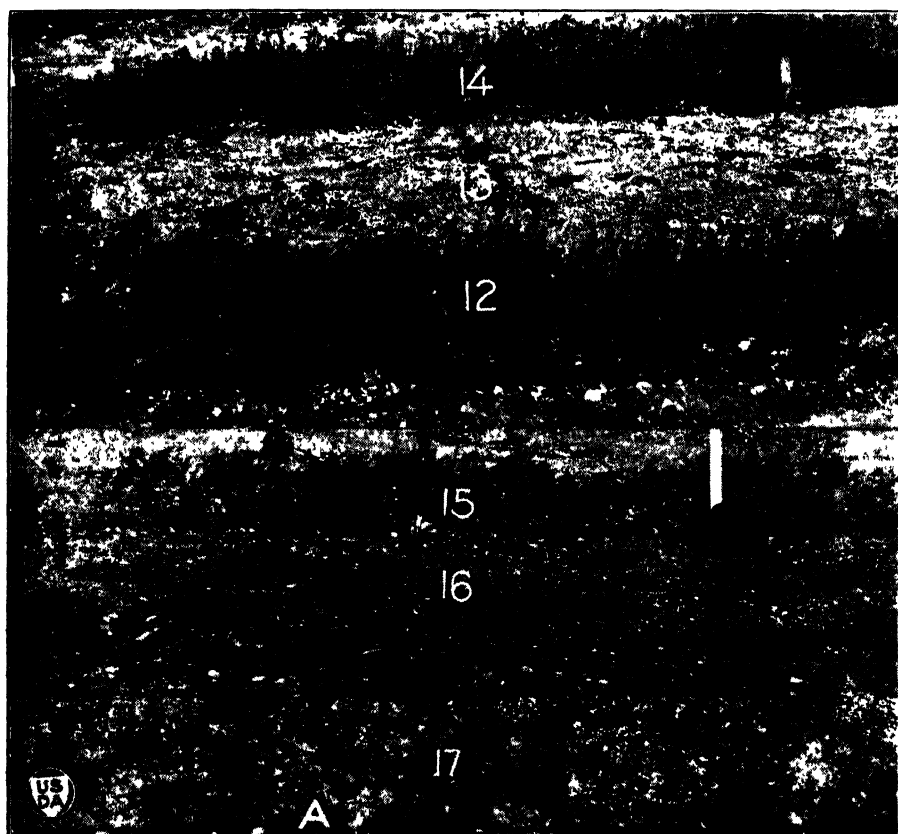
The disease can be controlled effectively in the nursery by placing a protective framework over the bed so that the full weight of the snow does not lie heavily on the trees, and press them down flat on the ground. It is by far the best and most efficient method of control which has been developed.

# **PLATE I**

**A.—Bed of 3-1 Norway spruce as it appeared in June, 1920, about two weeks after the snow had disappeared, showing the very high mortality and large amount of injury due to snow-molding fungi.**

**B.—Bed of Engelmann spruce as it appeared in June, 1920, about two weeks after the snow had disappeared, showing its relative freedom from snow-molding injury, as compared with Norway spruce.**





## PLATE 2

A.—Beds 12, 13, 14, 15, 16, and 17, 3-0 Douglas fir seedlings, as they appeared on May 27, 1921, about two weeks after the snow had disappeared. Bed 12 (control) received no special treatment, Bed 13 was mulched with timothy hay, Bed 14 was protected with a plank framework, Bed 15 was mulched with aspen leaves, Bed 16 was covered with canvas throughout the winter, Bed 17 was mulched with sterilized sand each autumn. Note the very poor stand on the mulched beds and the good stand and fine appearance of the protected bed (No. 14), as compared with the control bed (No. 12).

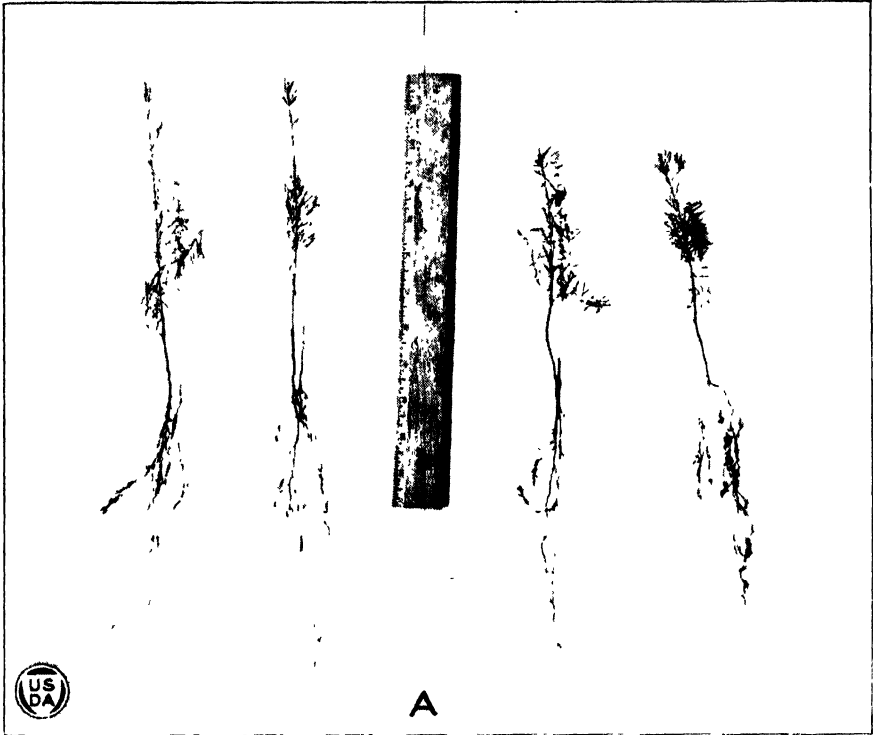
B.—The protective framework of 2 by 6's used on Bed 14, with a few of them removed from the front of the bed to show the 3-0 Douglas fir seedlings. Round logs may be used successfully instead of square timbers.



**PLATE 3**

A.—Healthy 3-o Douglas fir seedlings from Bed 14, protected by framework of 2 by 6 planks, in which there was practically no injury from snow-molding fungi.

B.—Three-year-old Douglas fir seedlings from Bed 12, unprotected, showing unhealthy appearance due to injury from snow-molding fungi.





# AN INFLUENCE OF MOISTURE ON BEAN WILT<sup>1</sup>

By LEWIS T. LEONARD<sup>2</sup>

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United States Department of Agriculture

In 20 years' experience with the inoculation of legumes there have arisen only two noticeable failures in field work which could not be attributed to soil or climatic conditions or to the lack of efficiency on the part of the culture involved. The first case occurred with cowpea, evidenced by normal growth in the plants from untreated seed and sickly plants from the inoculated cowpeas. The other failure occurred in conjunction with the inoculation of navy bean, *Phaseolus vulgaris*, on the farm of the Office of Forage Crop Investigations at Redfield, S. Dak., in 1920 and was brought to my attention by Messrs. R. A. Oakley and H. L. Westover of that office. In the latter case many of the plants from beans which had been treated with a pure liquid culture, such as is distributed by the Department of Agriculture, died during the growing season, and the damage was sufficiently extensive to be rather noticeable in comparison with the rows of plants from the seed planted in the dry condition. It was estimated that 90 per cent of the crop was killed among the treated beans, whereas the loss was very slight among the plants which were uninoculated. A duplicate experiment with two different cultures, made on different pieces of ground at Redfield in 1921, gave an average loss of 25 per cent, due to treatment with liquid culture.

The possibility that the bean legume organism assumes a definite pathological rôle was considered, but this theory has been completely discarded since the publication of the work of Miss Florence Hedges,<sup>3</sup> who isolated and described an entirely different organism as the cause of this disease. The material used by Miss Hedges was obtained at Redfield, S. Dak.

The elimination of the possibility first mentioned led to the consideration of a second theory regarding the prevalence of the disease—that is, that some constituent of the medium employed to grow the culture of legume bacteria exercises a stimulating influence on the pathogenic organisms already in or on the seed. Miss Hedges found that the causative organism of the disease in question was generally present in the seeds which were used in the experiment.

## EXPERIMENTAL PLAN

Attempts made to reproduce this diseased condition by the application of water and solutions containing the constituents of the media to beans which were planted on the Arlington Experimental Farm, Rosslyn, Va., gave practically no definite results in one year, despite

<sup>1</sup> Accepted for publication Sept. 2, 1922.

<sup>2</sup> A large amount of credit for the successful carrying out of this experiment is due to Mr. H. L. Westover and Mr. Samuel Garver of the Office of Forage Crop Investigations.

<sup>3</sup> HEDGES, Florence. A BACTERIAL WILT OF THE BEAN CAUSED BY BACTERIUM FLACCUMFACIENS NOV. SP. *Is Science*, N. S., V. 55, P. 433-434. 1922.

Observations on the roots revealed that nodules were quite generally absent and that their occasional occurrence seemed to be more closely connected to the presence of moisture in the soil than to the application of inoculating material.

#### CONCLUSION

It is indicated that a slight application of moisture either in the form of water, certain culture media in the broth form or legume bacteria culture will cause a stimulation of the bean wilt disease under conditions such as obtain at Redfield, S. Dak.

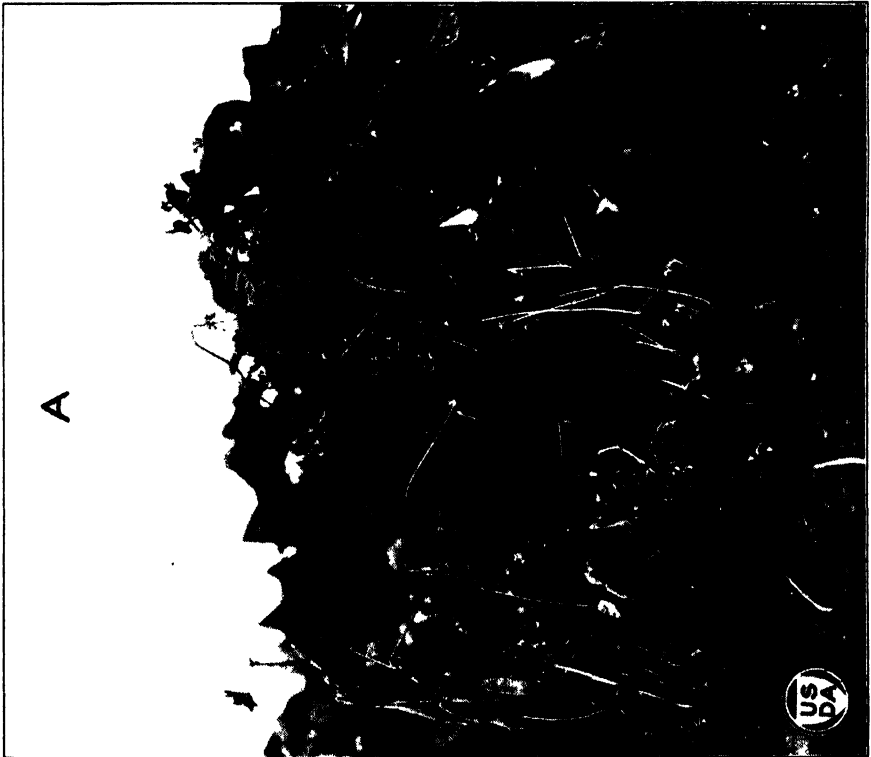
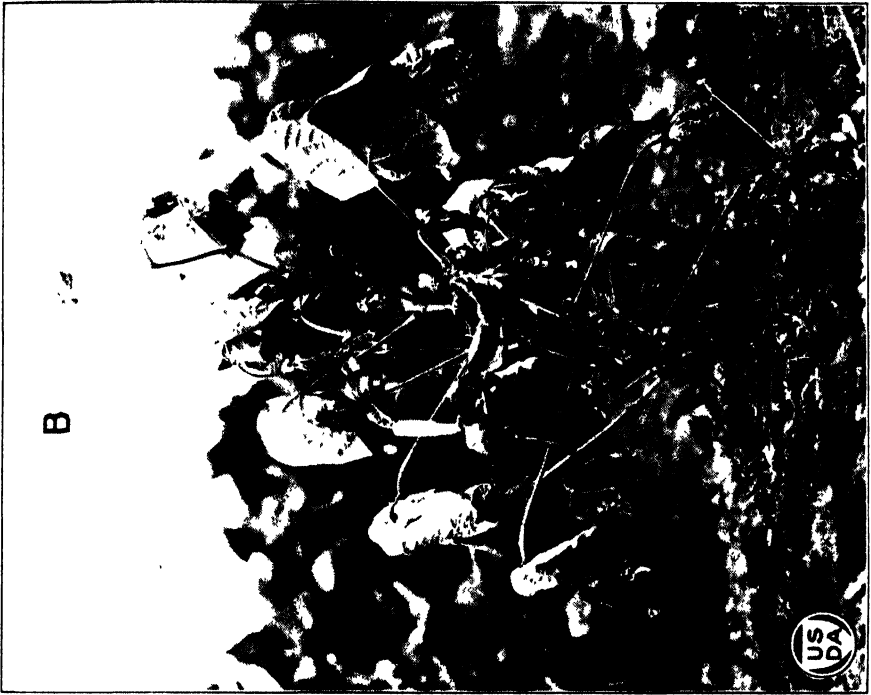
If the application of legume bacteria culture to beans is necessary, it is indicated that satisfactory results may be obtained by adding this material in the form of naturally or artificially inoculated soil in a dry condition at the rate of not less than 300 pounds per acre. If only a small amount of soil is available, it may be mixed with the seed and sown with them.

This stimulation of disease organisms is apparently not produced with similar treatment under the humid conditions and soils of the Arlington Farm, Rosslyn, Va.



**PLATE 1**

- A.—Plants in field showing symptoms of wilt beside a normal plant.**  
**B.—A plant showing the early stages of complete destruction of life by the inroads of the wilt disease organism.**







**PLATE 2**

A.—Plant in which disease has caused main shoot to wilt, but a side shoot is apparently unaffected.

B.—A completely destroyed bean plant which had formed pods.

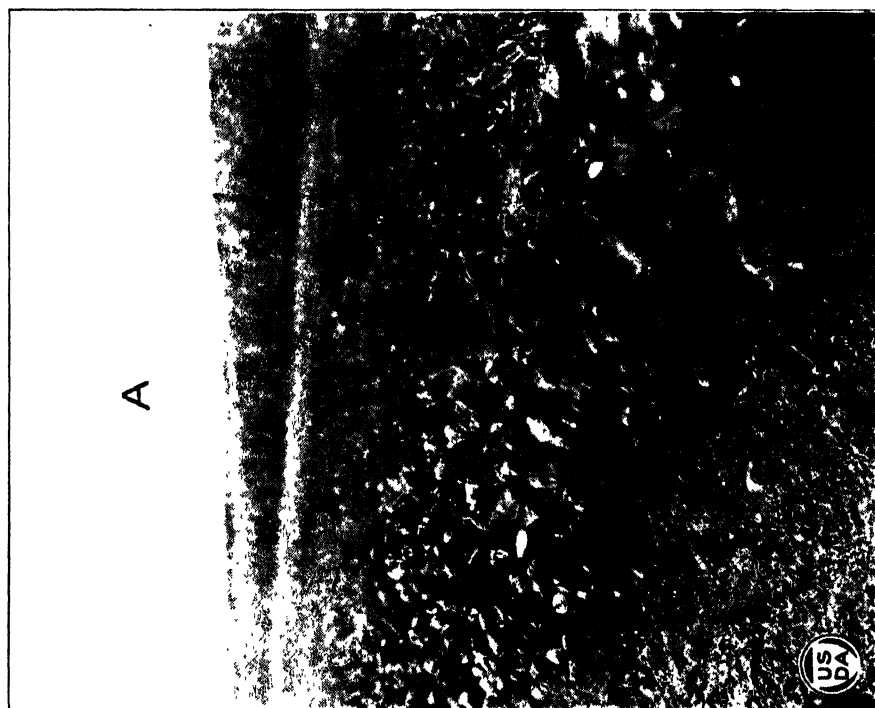
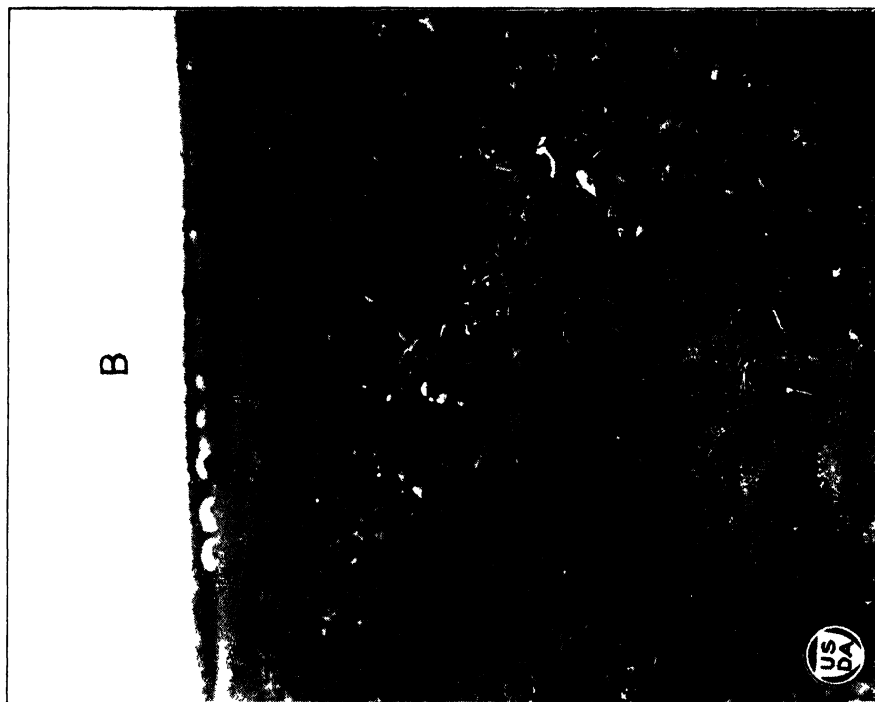
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**PLATE 3**

**A.—A part of a row of beans which were from seed planted dry.**

**B.—A part of a row of beans from seed which had been treated with Ashby broth.**

**White cloths indicate the location of wilted specimens.**





# THE PSEUDO-ANTAGONISM OF SODIUM AND CALCIUM IN DILUTE SOLUTIONS<sup>1</sup>

By H. S. REED, *Professor of Plant Physiology*, and A. R. C. HAAS, *Assistant Professor of Plant Physiology, College of Agriculture, University of California*

The effects of calcium and of sodium ions upon living organisms are so diverse that many physiologists have advanced the idea that these ions antagonize each other at the surface of the cell. Osterhout (6)<sup>2</sup> has suggested that antagonism depends upon the production of a union of NaCl and CaCl<sub>2</sub> with some constituent of the protoplasm, and that the surface may become saturated with the antagonizing salts. Below the saturation point it is claimed that the relative proportions of the salts will be of less importance than their total concentration. In other words, it is considered that no distinctly favorable ratios exist in solutions of low concentration.

An attempt to demonstrate antagonism between Na and Ca in dilute solutions was made by Breazeale and Reed (7). The results of their inquiry showed an apparent antagonism existing in solutions containing 230 parts per million of total solutes. At any rate, wheat plants made somewhat better growth in ratios of CaCl<sub>2</sub> and NaCl in which Osterhout had found the greatest amount of antagonism than in other ratios.

The large amounts of calcium found by Kelley and Cummins (5) in citrus plants, suggested the suitability of these plants as material for the further study of this problem. In the first experiment rough-lemon (*Citrus limonia*) seedlings were grown in solutions of NaCl and CaCl<sub>2</sub> containing the following mixtures (the pure solutions each being 0.004M) 100 Na:0 Ca; 0 Na:100 Ca.

The distilled water used in experiments 1 and 2 was found to be calcium free as determined by evaporating 500 cc. of the water to a small volume and testing for calcium.

Liter glass jars, each containing six seedlings, were filled with culture solution on April 23. The seedlings were supported in paraffined-cork stoppers in such a way as to exclude foreign matter from the culture jars. The solutions were renewed on May 5, 13, 24, and June 1. The cultures were grown out of doors under half shade (in a lath room about 3 feet high with sides of cheesecloth) in a south exposure. On June 7 the experiment was concluded. The seedlings were momentarily dipped into and well rinsed in distilled water in order to remove adhering solution and also to remove dust from the leaves. In preliminary experiments it had been found that 0.033 gm. of dust had accumulated on 39 rough-lemon seedlings weighing 14.928 gm. green weight, 2.950 gm. dry weight, and 0.233 gm. ash.

The seedlings were freed from adhering moisture by means of filter paper, and the green weight was obtained. The seedlings were dried to constant weight at 70° C. and were then ignited in a porcelain dish at low heat. The salts in the ash were extracted with hot water and the residue

<sup>1</sup> Accepted for publication Sept. 2, 1922. Paper No. 97, University of California, Graduate School of Tropical Agriculture, and Citrus Experiment Station, Riverside, Calif.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 757.

and the filters were then returned to the dish, dried, and ignited. The filtrate was added to the dish, evaporated to dryness and heated at low heat to constant weight in order to obtain the weight of ash. By means of dilute HCl, practically all of the ash was brought into solution, the residue being inappreciable.

TABLE I.—*Rough-lemon seedlings grown 45 days in solution cultures*

Ratio of Na and Ca in solution.	Number of seedlings analyzed.	Composition calculated to a basis of 100 plants.					
		Green weight.	Dry weight.	Ash.	Ca.	K.	Na.
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
100 Na:0 Ca.....	48	21. 83	5. 90	0. 2781	0. 0105	0. 0515	0. 0376
98 Na:2 Ca.....	48	26. 16	6. 83	. 3739	. 0335	. 0587	. 0420
0 Na:100 Ca.....	49	38. 95	8. 79	. 5873	. 1259	. 0609	. 0194

The results of the first experiment (Table I) show that better plants were obtained in cultures containing calcium; in fact, both green weight and dry weight increased where the amount of calcium was increased. In the pure NaCl solution the root tips appeared stunted and brown in color. The sodium content of the plants was slightly greater in cultures containing 98 Na:2 Ca than in pure NaCl solution. In this respect the results agree with those of Reed (7), though not in regard to the relative dry weight of plants produced.

Experiment 2 was begun April 27 and concluded June 7. The solutions were renewed at the same time as were those in experiment 1. Grapefruit (*Citrus grandis*) seedlings were used.

TABLE II.—*Grapefruit seedlings grown 42 days in solution cultures*

Ratio of Na and Ca in solution.	Number of seedlings analyzed.	Composition calculated to a basis of 100 plants.					
		Green weight.	Dry weight.	Ash.	Ca.	K.	Na.
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
100 Na:0 Ca.....	29	36. 29	8. 34	0. 4807	0. 0124	0. 0936	0. 0547
98 Na:2 Ca.....	38	29. 62	6. 34	. 4084	. 0161	. 0850	. 0470
0 Na:100 Ca.....	42	36. 20	7. 11	. 5195	. 0646	. 0948	. 0235

From the above table it appears that the 98 Na:2 Ca ratio had no superiority over the 100 Na:0 Ca as measured by weight of plants produced. This apparent contradiction to the foregoing experiment may be due to the fact that many of the seedlings in the pure NaCl solution died and were removed. Such seedlings were discarded, for it has been shown by Johnson (4) that absorption of ions may differ appreciably in living and in dead cells. This left only the best plants in this lot; consequently we are dealing with what may be regarded as an inherently superior lot of individuals, while in the other cases we are dealing with the entire population, weak and strong.

In experiment 3, which was begun on May 4 and concluded on May 27, rough-lemon seedlings were grown in solutions of NaCl and CaCl<sub>2</sub>. This

experiment was concluded early in order to avoid the possible killing of root tips. Even by so doing many of the seedlings in the solution having a ratio of 100 Na:0 Ca had dead root tips and were, therefore, discarded.

TABLE III.—*Rough-lemon seedlings grown 23 days in solution cultures*

Ratio of Na and Ca in solution.	Number of seedlings analyzed.	Composition calculated to a basis of 100 plants.				
		Green weight.	Dry weight.	Ash.	K.	Na.
		Gm.	Gm.	Gm.	Gm.	Gm.
100 Na:0 Ca.....	28	23. 47	4. 27	0. 236	0. 0414	0. 0272
98 Na:2 Ca.....	40	26. 94	4. 64	. 273	. 0532	. 0225
85 Na:15 Ca.....	62	24. 56	4. 13	. 263	. 0446	. 0157

The results of this experiment are quite at variance with those of the preceding experiments. Here the differences in green weight and dry weight are so small as to be attributable to the inherent variability of the plants rather than to differences in the composition of the solutions.

Three experiments were conducted with the same mixtures as in experiment 2, using 60 seedlings for each ratio. African sour-orange (*Citrus aurantium*), grapefruit (*C. grandis*), and St. Michael orange (*C. sinensis*) seedlings were used, the solutions being renewed at the end of two weeks. After four weeks had elapsed the experiments were discarded because the root tips of the seedlings in the solution with the ratio 100 Na:0 Ca were dead.

On June 25 rough-lemon seedlings were placed in mixtures similar to those in experiment 3. On July 8 the solutions were renewed and on July 9 the experiment was discarded. In the solution having the ratio 100 Na:0 Ca the root of nearly every seedling had become slimy and gelatinous and was dead for a considerable distance back from the root tip. The roots of the seedlings in the solutions having the ratios 98 Na:2 Ca and 85 Na:15 Ca, respectively, were bright in appearance and although small had begun to develop lateral rootlets.

The above experiments indicate that citrus seedlings can not survive many days in a solution of 230 parts per million NaCl (0.004 M; 100 Na:0 Ca). One might be led to believe that antagonism has occurred with the ratio 98 Na:2 Ca, since the seedlings are found to grow when a trace of calcium is present, whereas, in the solution with a 100 Na:0 Ca ratio they are soon stunted and shortly afterward die. Experiment 4 indicates, however, that this is an instance of calcium starvation rather than of NaCl toxicity. It was found by analyses of culture solutions that citrus seedlings do not require a large concentration of calcium provided the supply is maintained. The usual methods of chemical analysis are not sufficiently delicate to detect significant absorption differences in the brief periods required by the conductivity method of studying permeability.

Experiment 4 was carried on with St. Michael orange seedlings, which were placed in the culture jars on September 4. Each set of cultures consisted of 12 jars with 3 seedlings per jar in the first set and 2 seedlings per jar in the remainder.

The first culture solution was that employed by Hoagland (3). The cultures were still growing on November 1. The second set of cultures



was grown in Hoagland's nutrient solution in which the calcium had been replaced by potassium; 500 cc. of this solution was evaporated to a small volume and was then tested and found to be calcium-free. Within 3 days after the experiment was begun, the root tips of the seedlings in the calcium-free cultures were becoming brown and failed to show any evidence of growth. After 7 to 10 days the root tips of most of the seedlings were becoming slimy and gelatinous. At the end of 2 weeks most of the roots appeared to be dead. The full amount of calcium of Hoagland's nutrient solution was then added in the form of calcium nitrate to six of the jars. Within a few days many of the seedlings began to recover, although several of the seedlings in advanced stages of decomposition failed to respond to the addition of the calcium nitrate.

In Plate 1, A, B, are shown various stages in the progressive killing of the primary root, beginning with the root tip and proceeding toward the cotyledons. Plate 1, B, shows some of the roots of Plate 1, A, somewhat enlarged. (The material closely held by the roots is composed of small pieces of sphagnum from the germination box.) It will be seen that when calcium was added to the solution, laterals were developed, the lowermost lateral always sharply separating the living from the dead portions of the primary root. Frequently in the absence of calcium the primary root was unable to grow. In such cases, when calcium was added, a lateral rootlet was produced immediately back of the root tip and this lateral then assumed the position of the primary root (Plate 1, A, B). One of the most characteristic effects of a lack of calcium is the gelatinization of the surface layers of the root. This process is most active in the apical region of the root and progressively decreases in intensity toward the upper portion. As time goes on the gelatinization proceeds inward until finally the root becomes translucent.

In the initial stages of this process of gelatinization, recovery is possible if a suitable amount of a calcium salt is added. The root then regains its firm white appearance and functions properly. In advanced stages of gelatinization recovery does not follow the addition of a calcium salt, although new laterals may be produced from the upper portion of the root. Herbst (2), Hansteen Cranmer (1), and others have observed a similar gelatinization in other organisms in the absence of calcium and likewise have observed recovery when calcium salts were added.

There is thus evidence that the stunted appearance of roots in pure NaCl solutions above discussed is not due to the toxicity of NaCl but to the lack of calcium. Further study of this factor was made in which more concentrated solutions of NaCl, having toxic properties, were used. Seedlings placed in Hoagland's solution minus calcium failed to develop although it contained but 30 parts per million NaCl. They speedily recovered and grew when  $\text{CaCl}_2$  was added. Another series in calcium-free Hoagland's solution plus 1,000 parts per million NaCl likewise failed to develop. When  $\text{CaCl}_2$  was added to this latter solution the plants made good growth in spite of the amount of NaCl present.

It appears, therefore, that the harmful effect of 100 Na:0 Ca in dilute solutions is not due to the lack of ions which antagonize the sodium, but rather to calcium starvation, and that the result is conspicuous in the case of a plant like citrus which is extremely sensitive to calcium. In solutions containing only 0.004 M. NaCl, it seems improper to speak of a toxic action upon the roots. (Cf. True, 8.)

In the study of the antagonism of Na and Ca with citrus seedlings it seems impossible to utilize solutions as dilute as those commonly employed as nutrients on account of the starvation which results when the calcium is reduced to extremely low concentrations. In other words, the assumption that no distinctly favorable ratios exist in solutions of low concentration is supported.

#### SUMMARY

Citrus seedlings grown in absence of calcium soon show injury to the root system, as evidenced by the gelatinization of the superficial layers and the ultimate death of the root. The tops, however, may not show the effect for some time after the injury to the roots has become severe.

If the injury has not progressed too far, the addition of calcium to cultures in which the roots are gelatinized induces the production of lateral rootlets the lowermost of which definitely delimits the dead from the living portion of the root.

The varieties of citrus here studied are known to possess a marked capacity for the absorption of calcium ions. Hence in very dilute solutions the amounts present may be too far below the equilibrium point within the plant to avoid a condition of starvation. In such cases we are dealing with the phenomenon of starvation rather than that of antagonism. These experiments with citrus seedlings have not demonstrated the existence of antagonism between sodium and calcium when the plants are grown in very dilute solutions.

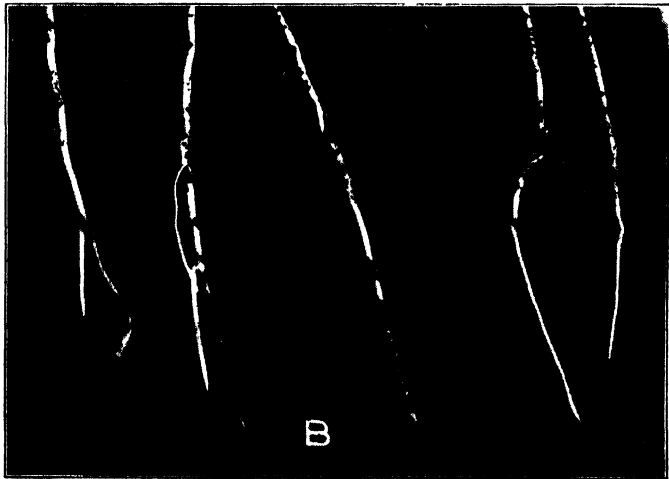
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# **PLATE 1**

A.—Orange seedlings grown in calcium-free Hoagland's solution. At the end of two weeks the full amount of calcium of Hoagland's solution was added in the form of calcium nitrate. The point at which recovery occurred is seen on each root.

B.—Showing the point of recovery of roots of orange seedlings (Pl. 1, A) when calcium is added to the solution after two weeks' exposure to calcium-free Hoagland's solution.





# INFLUENCE OF THE HYDROGEN-ION CONCENTRATION ON THE GROWTH AND FIXATION OF NITROGEN BY CULTURES OF AZOTOBACTER<sup>1</sup>

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Investigations in this laboratory have shown that the presence or absence of *Azotobacter* in natural soils is very closely associated with, if not dependent upon, the absolute reaction of the soil solution (4).<sup>2</sup> It has also been shown that in the laboratory the presence of this group of organisms in a soil can be controlled by varying the hydrogen-ion concentration of the soil (5).

In connection with these investigations it seemed desirable to study the influence of the hydrogen-ion concentration of laboratory culture media upon the growth and nitrogen fixing ability of pure cultures of *Azotobacter*.<sup>3</sup>

In their original investigations on *Azotobacter*, Beijerinck and Van Delden (1) failed to secure appreciable fixation of nitrogen by pure cultures. On the other hand, Lipman (6) experienced no difficulty in securing marked fixation by pure cultures, and he presents data to show that the ability of pure cultures to fix nitrogen in the media usually employed for their culture depended upon the neutralization of the acidity arising from the potassium phosphate present. Lipman further demonstrated that, quantitatively, the nitrogen fixed by pure cultures was inversely proportional to the titratable acidity of the media, and called attention to the probability that Beijerinck's failure to secure nitrogen fixation was due to the unfavorable reaction of the medium employed. Since Lipman's work appeared, it has been universally accepted that these organizations will not function in a high concentration of acid.

With regard to the effect of different degrees of acidity, or hydrogen-ion concentrations, upon the growth of pure cultures of this group of organisms little information has been published. Fred (3) observed the growth of two different cultures in a medium of varying hydrogen-ion concentrations and noted no growth at  $P_H$  6.4 to 6.6, while growth occurred at  $P_H$  6.6 to 6.8. On the other hand, when grown in a medium of  $P_H$  7.2 the final hydrogen-ion concentration was found to be  $P_H$  5.1. As already mentioned, previous investigations in this laboratory indicated that the hydrogen-ion concentration of the soil solution is the major factor in controlling the presence of this group of organisms in soils. Also, that the maximum concentration of hydrogen ions tolerated by this group of

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 767.

<sup>3</sup> The term "pure culture" is here used in a restricted sense. Owing to the extreme morphological variations exhibited by this group of organisms it is frequently very difficult to ascertain with certainty the purity of a given culture. All cultures used in these experiments conformed to the description given in the text of this paper.

organisms appear to be very close to  $P_H$  5.9 to 6.0. It seemed of interest, therefore, to ascertain the effect of varying the hydrogen-ion concentration of laboratory media upon the activity of a number of strains of *Azotobacter*.

#### METHODS EMPLOYED

Crude cultures of *Azotobacter* were prepared from a number of soils by inoculating a mannite or dextrose cultural solution and incubating until a characteristic *Azotobacter* film developed. Portions of these films were then streaked upon mannite or dextrose agar and the streaking process repeated from isolated colonies until all colonies developing were similar and yielded, when stained, only typical *Azotobacter* cells. No effort was made to identify the various cultures.

The medium employed had the following composition: Monobasic potassium phosphate, 0.2 or 5.0 gm.; magnesium sulphate, 0.2 gm.; sodium chlorid, 0.5 gm.; ferric chlorid, 2 drops of a 10 per cent solution; dextrose or mannite, 20.0 gm.; distilled water 1,000 cc. Flasks of this medium, slightly alkaline to brom-thymol-blue, were inoculated from an agar streak culture and aerated vigorously at 28° C. until heavily clouded (two to four days). The cultures were again stained and examined, and all flasks showing morphological forms other than typical *Azotobacter* were discarded. One to five per cent of such a culture was used as an inoculum.

The quantity of sterile media necessary to set up an experiment was prepared and inoculated. Fifty cubic centimeter quantities were measured aseptically into carefully washed sterile 300 cc. Erlenmeyer flasks. The quantity of NaOH or HCl necessary to adjust the 50 cc. of media to approximately the desired reaction was determined and this quantity, previously sterilized, was carefully added to four flasks. The hydrogen-ion concentration of the contents of one of these flasks was immediately determined and recorded as the initial reaction. Hydrogen-ion determinations were made colorimetrically, a control being occasionally run electrometrically. A small quantity of formaldehyde was added to a second flask to act as a control with which to compare growth and nitrogen fixation. The remaining two cultures were incubated at 28° C. for two weeks. The quantity of acid or alkali required to produce a given change in reaction varied only slightly from time to time.

In the earlier experiments, where only 0.02 per cent  $KH_2PO_4$  was used, the buffer index was very low and necessitated the use of very dilute (N/80) NaOH in adjusting the reaction. Where 0.5 per cent  $KH_2PO_4$  was used (experiments 16 to 20 inclusive) the buffer index was much higher, requiring approximately ten times as much acid or alkali to produce a given change in reaction, and N/10 NaOH was used to correct the reaction.

During incubation the cultures were examined at frequent intervals and the presence or absence of growth recorded. The formaldehyde produced a slight change in the physical appearance of the medium in the control flasks and rendered it somewhat difficult to detect very slight changes in the appearance of cultures. Where it was impossible to determine definitely whether growth had taken place it has been indicated in Tables I and IV with a question mark.

After two weeks' incubation a final examination for growth was made, the hydrogen-ion concentration of the cultures recorded, and the total nitrogen present determined by the Kjeldahl method. The quantity of

nitrogen in the control flasks and in the cultures where no growth was visible was very small and, as will be observed in Table II, the experimental error for the controls was large. In examining the data relative to the quantity of nitrogen fixed this experimental error must be taken into consideration, otherwise erroneous conclusions are likely to be drawn. In the data recorded in Tables II and IV the quantity of nitrogen present in controls has been deducted from that present in the inoculated flasks and only the net gain recorded. Nitrogen determinations were made on at least four control flasks to determine the probable error where only small quantities of nitrogen were present.

#### INFLUENCE OF REACTION UPON GROWTH

In Table I data are recorded relative to the influence of the hydrogen-ion concentration of the culture medium upon the growth of several strains of *Azotobacter*. A study of these data will show that the maximum acidity permitting appreciable growth to take place is very close to  $P_H$  5.9. There is only one instance where definite growth is recorded in a higher degree of acidity (experiment 15,  $P_H$  5.8) and the duplicate of this culture failed to show growth. Another strain, 12-323-6, not cultured at  $P_H$  5.9 in the experiment here recorded grew at  $P_H$  5.9 in an unrecorded experiment. All cultures showed some growth at  $P_H$  5.9-6.0. The amount of growth that took place at  $P_H$  5.9 was in most instances very slight, and growth increased as the acidity decreased until  $P_H$  6.1 to 6.3 was reached. At this reaction growth appeared in most instances to be as vigorous as at lower hydrogen-ion concentrations. Cultures 407-5 and 449 did not grow as abundantly as did the other cultures, even when the reaction was favorable. These two cultures failed to produce a surface film, and when growth is compared with nitrogen fixation it will be noted that they also fixed small quantities of nitrogen.



TABLE I.—The influence of the hydrogen-ion concentration of culture media upon the growth of *Asotobacter*

Experiment No.	Culture.	Initial reaction expressed as P <sub>H</sub> (growth records for 2 weeks).																	Ca Co.
		Below 5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.2	Above 7.6
6	456-21	—	—	—	—	—	+	++	+++	+++	—	—	—	—	—	—	—	—	—
8	C	—	—	—	—	—	—	+	+++	+++	—	—	—	—	—	—	—	—	—
9	19-399	—	—	—	—	—	+	—	—	+	+++	+++	+++	+++	—	—	—	—	—
11	456-21	—	—	—	—	—	+	+	+++	—	—	—	—	—	—	—	—	—	—
12	40-399	—	—	—	—	—	+	+	—	—	—	—	—	—	—	—	—	—	—
13	12-323-6	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—
14	C	—	—	—	—	—	—	+	+++	—	—	—	—	—	—	—	—	—	—
15	40-399	—	—	—	—	—	—	+	+++	—	—	—	—	—	—	—	—	—	—
16	407-5	—	—	—	—	—	—	+	+++	—	—	—	—	—	—	—	—	—	—
17	14-399-6	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—
18	E	—	—	—	—	—	—	+	+++	—	—	—	—	—	—	—	—	—	—
19	407-5	—	—	—	—	—	—	+	+++	—	—	—	—	—	—	—	—	—	—
20	499	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—

— No visible growth.  
 ? Questionable growth.  
 + Slight but distinct growth.  
 ++ Good growth, but no film.  
 +++ Heavy growth and film formation.

## INFLUENCE OF REACTION UPON NITROGEN FIXATION

Data relative to the influence of the hydrogen-ion concentration of the culture medium upon the fixation of nitrogen by cultures of Azotobacter are recorded in Table II. In examining these data the rather large experimental error for the controls of the individual experiments should be noted. In no instance is there recorded a definite fixation of nitrogen in a hydrogen-ion concentration greater than  $P_H$  5.9. The data for  $P_H$  5.9, 6.0, and 6.1 are inconclusive, the quantities of nitrogen fixed, if any, being small. At  $P_H$  6.2 and above definite fixation occurred, the maximum in most experiments being recorded at  $P_H$  6.3 to 6.5. Vigorous fixation of nitrogen appeared to be associated with the growth of a definite film on the surface of the medium.

TABLE II.—The influence of the hydrogen-ion concentration of culture media upon the fixation of nitrogen by Azotobacter

Experiment No.		Culture.	Initial reaction expressed as P <sub>2</sub> nitrogen fixed per culture of 50 cc. media (mgm.).																	Probable error controls.							
			Below 5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.8	6.9	7.2	Above 7.6		CaCO <sub>3</sub> .						
11		426-21			0.08					0.14	0.17								4.81		5.18		4.84				±0.51
12		40-399	0.00		0.00				0.43	0.00	1.81								5.27		5.27						±0.51
13		12-23-6	0.37		0.74				0.00	1.11																	±0.95
14		C	0.00		0.18				0.00	1.02																	±0.46
16		407-5	0.00		0.00				1.35	0.51	1.16																±0.46
17		14-399-6	0.00		0.32				0.60	0.60	0.10																±1.01
18		E	0.28		0.19				0.47	0.47																	±0.45
19		407-5	0.10		0.21				0.31	0.57	0.62																±0.43
20		499	0.00		0.17				0.17	1.12	0.41																±0.43
Average....			0.00		0.00				0.20	1.63	0.92																±0.77
			0.00		0.22				0.00	0.00	0.32																±0.05
			0.14		0.00				0.47	0.10	0.73																±0.95
			0.50		0.00				0.00	1.07	1.89																±0.64
			0.31		0.21				0.63	1.58	1.26																±0.64
			0.12		0.11				0.40	0.66	0.77																±0.27
					0.23				0.14		1.33																±0.27
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## CHANGES IN REACTION PRODUCED BY GROWTH

The initial and final reactions of the cultures are recorded in Table III. The medium used in all experiments below 16 contained only 0.02 per cent  $\text{KH}_2\text{PO}_4$  and possessed a very low buffer index. The quantity of N/1 base or acid necessary to produce a change of 0.1  $\text{P}_\text{H}$  in 100 cc. of this medium was only approximately 0.006 cc. In these experiments some rather marked changes in reaction were recorded. The changes sometimes indicated the production of acid, at other times the production of basic compounds. However, when calculated in terms of total acid or base the quantities are insignificant.

TABLE III.—The influence of the growth of *Azotobacter* in culture media upon the reaction of the media

Experiment No.	Culture.	Initial reaction expressed as P <sub>m</sub> , after a weeks' growth.																		Per cent KH <sub>2</sub> PO <sub>4</sub> .
		Below 5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.2	Above 7.6	
6	426-21	5.4—	5.4—	5.6	5.6	5.8	5.8	5.9	6.1	6.0		5.8								a 0.02
		5.5	5.4	5.7	5.7	5.8	5.9	6.0	6.1	6.2		6.4								0.02
		5.4	5.4	5.8	5.8		5.9				6.1		6.1		6.8	6.8				0.02
7	12-23-6																			0.02
																				0.02
8	C																			0.02
																				0.02
9	426-21																			0.02
																				0.02
10	40-399																			0.02
																				0.02
11	12-23-6																			0.02
																				0.02
12	C																			0.02
																				0.02
13	407-5																			0.02
																				0.02
14	24-399-6																			0.02
																				0.02
15	C																			0.02
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16	407-5																			0.02
																				0.02
17	24-399-6																			0.02
																				0.02
18	C																			0.02
																				0.02
19	407-5																			0.02
																				0.02
20	449																			0.02
																				0.02

a Media containing 0.02 per cent  $\text{KH}_2\text{PO}_4$  required only 0.006 cc. N/1 NaOH per 100 cc. to change the  $\text{P}_\text{H}$  0.1.

b Media containing 0.50 per cent  $\text{KH}_2\text{PO}_4$  required 0.05 cc. N/1 NaOH per 100 cc. to change the  $\text{P}_\text{H}$  0.1.

These results led to the conclusion that no satisfactory data as to changes in reaction could be secured in a medium so poorly buffered. The quantity of  $\text{KH}_2\text{PO}_4$  was accordingly increased to 0.5 per cent. Higher concentrations of phosphate were sometimes found to be toxic. This increase in phosphate increased the buffer effect approximately ten times, the media containing 0.5 per cent requiring 0.05 cc. N/1 NaOH to effect a change of 0.1  $P_H$  in 100 cc. The buffer effect was still low, but an examination of experiments 16 to 20 will show that changes in  $P_H$  produced by the growth of the various cultures were practically eliminated. If acid or basic metabolic by-products are produced under these experimental conditions the quantities are inappreciable.

Fred (3) recorded a change in  $P_H$  from 7.2 to 5.1. The media employed by him, however, contained only 0.02 per cent phosphate, and the actual quantity of acid necessary to produce the recorded change in  $P_H$  was probably very small. Stoklasa (7) recorded the production of as high as 3.3 cc. N/1 acid per 100 cc. media. This quantity would have produced very marked changes in the  $P_H$  of our media. However, the purity of Stoklasa's cultures has been questioned by Bonazzi (2).

In Table IV are recorded in parallel columns the initial reaction, growth, final reaction, and milligrams of nitrogen fixed for four experiments. This table is included in order that these various factors may be compared one with the other without the necessity of examining several tables. In figure 1 the influence of the reaction on nitrogen fixation is shown graphically.

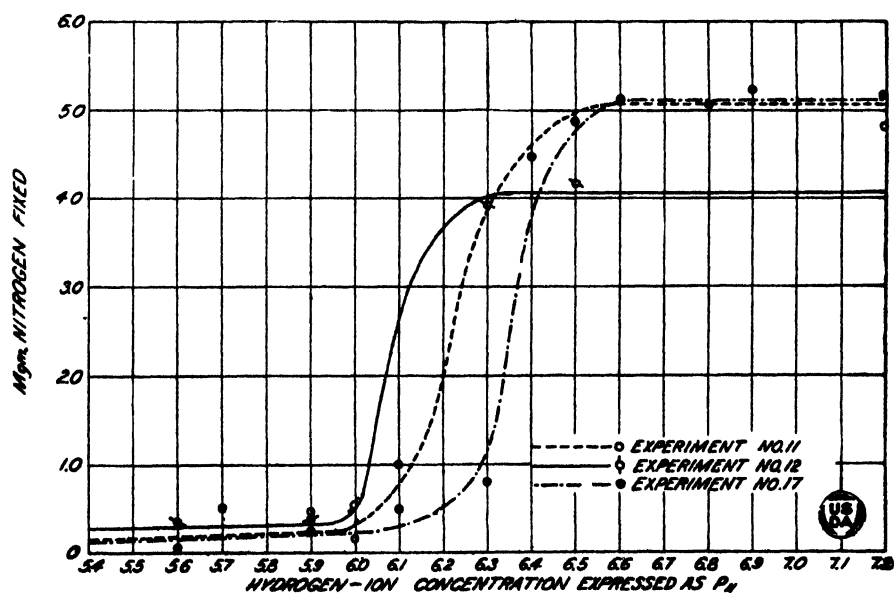


FIG. 1.—Effect of hydrogen-ion concentration on nitrogen fixation.

TABLE IV.—Comparison of initial reaction, growth, final reaction, and nitrogen fixed by four cultures of *Azotobacter*

Experiment 11, Culture 416-21.				Experiment 12, Culture 40-399.				Experiment 17, Culture 14-399-6.				Experiment 28, Culture E.			
Initial P <sub>n</sub> .	Growth.	Final P <sub>n</sub> .	Nitrogen fixed.	Initial P <sub>n</sub> .	Growth.	Final P <sub>n</sub> .	Nitrogen fixed.	Initial P <sub>n</sub> .	Growth.	Final P <sub>n</sub> .	Nitrogen fixed.	Initial P <sub>n</sub> .	Growth.	Final P <sub>n</sub> .	Nitrogen fixed.
5.6	—	5.4—	Mgm. 0.08	5.4—	—	5.4—	Mgm. 0.00	5.4—	—	5.4—	Mgm. 0.00	5.4—	—	5.4—	Mgm. 0.00
5.6	?	5.4—	.00	5.4—	—	5.6—	.37	5.4—	—	5.4—	.10	5.4—	—	5.4—	.00
5.9	—	5.6	.43	5.6	?	5.7	.00	5.7	?	5.6	.21	5.7	—	5.8	.17
5.9	?	5.6	.43	5.7	?	5.7	.74	5.8	?	5.8	.82	5.7	—	5.6	.00
6.0	?	5.4—	.00	5.9	+	5.7	.83	5.9	+	5.9	.31	5.9	+	5.9	.17
6.1	?	5.4—	.17	5.9	+	5.7	.00	5.9	+	5.8	.21	5.9	+	5.9	.20
6.1	+	5.5	1.81	6.0	+	5.8	1.11	6.1	+	6.1	.62	6.0	+	6.4	1.12
6.4	+	6.7	4.12	6.3	+	5.7	.00	6.3	+	6.0	.41	6.1	+	6.4	1.63
6.4	+	6.7	4.75	6.3	+	6.5	3.79	6.3	+	6.1	.93	6.1	+	6.1	.41
6.5	+	5.4—	2.94	6.3	+	6.5	4.06	6.3	+	6.1	.73	6.1	+	6.1	.92
6.5	+	6.4	2.85	6.5	+	5.6	3.70	6.5	+	6.2	4.04	6.2	+	6.1	1.43
6.6	+	7.3	4.81	6.5	+	6.4	4.62	6.5	+	6.6	5.06	6.3	+	6.3	1.22
6.6	+	7.3	5.44	7.6	+	5.7	3.97	6.8	+	6.7	5.37	6.3	+	6.3	1.12
6.9	+	7.3	5.18	7.6	+	5.9	3.98	Ca CO <sub>3</sub>	+	6.7	4.75	6.3	+	6.2	1.43
6.9	+	7.5	5.27	—	—	—	—	Ca CO <sub>3</sub>	+	—	5.59	6.8	+	6.6	1.84
7.2	+	7.4	4.84	—	—	—	—	—	+	—	4.73	6.8	+	6.6	1.63

— No visible growth.

? Growth indefinite.

+ Slight but distinct growth.

++ Good growth, with no film formation.

++++ Heavy growth, with film formation.

## SUMMARY

The data presented in this paper point very definitely to a limiting hydrogen-ion concentration of  $P_H$  5.9 to 6.0 for the various cultures of *Azotobacter* employed when grown under the conditions of these experiments. Vigorous growth and nitrogen fixation took place at  $P_H$  6.1 to 6.5, the optimum  $P_H$  for nitrogen fixation apparently being somewhat higher than the optimum for growth. Very slight, if any, changes in the reaction of the media are produced by the growth of the various strains of *Azotobacter* studied, indicating the production of inappreciable quantities of acid or basic metabolic by-products.

The data for pure cultures here presented agree very closely with our findings in soil and tend to substantiate former conclusions that this group of organisms will not exist and function in soils, the hydrogen-ion concentration of which is greater than  $P_H$  5.9 to 6.0.

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43323-23—3



# SUNFLOWER INVESTIGATIONS<sup>1</sup>

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## INTRODUCTION

The value of sunflowers for a silage crop in the Pacific Northwest has become quite well established. Their resistance to drought and early frosts, and the fact that they yield a large tonnage of green material per acre, justify a more complete knowledge of the proper spacing of plants in the row and the best stage of cutting the plant for silage purposes. With these facts in mind, an investigation was planned which would yield data on the proper method of planting and the proper time of harvesting.

The composition of the sunflower plants was first studied when harvested at various stages during growth, and when grown under two different spacings, namely, 4 to 8 inches, and 36 inches, apart in the row. The sunflower plants grown under the two systems of planting were compared for the percentages of leaves, stalks, stems, and flowers, and analyses were made of composite samples of each of these plant parts. Not only was the composition of the sunflowers determined in these two systems of planting and at various stages of maturity, but also the composition of the silage made from the sunflowers cut at each of these stages of maturity and from each of the two different spacings of plants in the row.

## PREVIOUS WORK

Shaw and Wright,<sup>2</sup> of the United States Department of Agriculture, published data on the composition of sunflowers grown at the Dairy Division Experiment Farm at Beltsville, Md. Their investigation included the composition of sunflowers cut at seven different stages of maturity.

Blish,<sup>3</sup> of the Montana Experiment Station, has recently published data on the effect on the composition and quality of silage of cutting sunflowers at different stages of growth. All results showed that silage of good quality resulted from sunflowers cut at the different stages of maturity selected by him. The problems, then, that present themselves in Idaho are: First, what is the most favorable stage of maturity for harvesting the sunflowers for silage purposes, and, second, what is the proper distance apart of planting in order that the maximum feeding value may be secured?

## EXPERIMENTAL WORK

The sunflowers used in this investigation were grown at the Idaho State Experiment Station at Moscow, Idaho, in the summer of 1920, by officials of the agronomy department, who kindly allowed us to sample the

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<sup>2</sup> SHAW, R. H., and WRIGHT, P. A. A COMPARATIVE STUDY OF THE COMPOSITION OF THE SUNFLOWER AND CORN PLANTS AT DIFFERENT STAGES OF GROWTH. *In Jour. Agr. Research*, v. 20, p. 787-793. 1921. Literature cited, p. 792-793.

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sunflowers at all stages of maturity. Additional work on the 1921 crop of sunflowers is included later in this paper.

The plants were grown from seed of the Giant Russian variety on Palouse silt loam soil. The plants grew luxuriantly, many of the largest of them reaching a height of from 10 to 12 feet.

#### STAGES OF MATURITY SELECTED

In selecting the proper stages at which to sample the sunflowers considerable difficulty was encountered in choosing definite stages of growth, since the sunflower plant does not offer any definite and sharply defined differences at any time during its growth. After due consideration, the stages were arbitrarily chosen in terms of budding and flowering in the early period of growth and, later on, of the degree of hardness of the seeds in the main or top flower. Five stages were selected, as follows: First, when the first bud was appearing on the top of the plant; second, when the first flower was about 3 inches in diameter but no seed had developed; third, just before the seeds of the first flower were in the dough stage; fourth, when the seeds of the first flower were well into the dough stage and the rays were just beginning to fall; fifth, when the seeds of the first flower were quite hard and its rays had fallen.

#### METHOD OF SAMPLING

Representative samples were secured by collecting a number of plants of uniform development and weighing and measuring each plant; the average was then recorded. Composite samples of sufficient size were made by cutting in a small silage cutter the constituent plants and then mixing thoroughly. A portion of each sample was dried in an electric oven to secure the percentage of moisture. A second portion was air dried and reserved for the approximate analysis. The remaining portion was used for making silage. For this purpose quart milk bottles were tightly filled and each stoppered with a rubber stopper containing a bent glass tube which had its outlet in a beaker of mercury. This arrangement allowed the fermentation gases to escape but prevented air from gaining access to the silage. This procedure was found to be very satisfactory and in every case resulted in a good quality of silage.

#### DIFFERENT SPACINGS OF PLANTS

In the early stages of growth, when the plants were about 6 inches high, the plants were thinned in order to secure data on the yield and composition of sunflowers when grown at various distances apart in the row.

In order to secure chemical data, two distances apart were chosen, which represented as nearly as possible the extremes of planting and also the two extreme types of sunflowers.<sup>4</sup> The two types selected were plants grown 36 inches apart and plants grown from 4 to 8 inches apart. The former system resulted in plants with an abundance of leaves and flowers on one large stalk, while the latter resulted in rather tall, spindly stalks with much smaller leaves and flowers. The data on the composition of sunflowers and sunflower silage are given in Table I.

<sup>4</sup> The spacings of plants intermediate between 4 inches to 8 inches and 36 inches were not included in this investigation, as the analytical work would have been enormously increased, and it was not certain that the results would show differences sufficient to warrant the additional analyses.

TABLE I.—Composition of sunflowers and sunflower silage (1920)

Stage of growth.	Average height.	Average weight.	Distance apart in rows.	Moisture.	Anhydrous material.	Crude protein.	Ether extracted.	Crude fiber.	Nitrogen-free extract.	Ash.	Type of material analyzed.
	<i>Fl. m.</i>	<i>Lbs. oz.</i>	<i>Inches.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	
Budding.....	8 10	13 2	36	84.45	15.55	15.82	1.95	24.07	45.80	12.36	Original.
Do.....	8 10	13 2	36	86.00	14.00	18.06	1.68	21.55	43.88	13.93	Silage.
Do.....	9 2	3 13	4 to 8	84.25	15.75	8.82	2.01	26.68	50.37	12.12	Original.
Do.....	9 2	3 13	4 to 8	86.35	13.65	11.56	1.32	23.99	48.73	14.40	Silage.
First flower in blossom.....	9 10	9 9	36	84.15	15.85	12.20	2.39	20.25	52.40	12.76	Original.
Do.....	9 10	9 9	36	85.00	15.00	15.93	3.13	18.32	48.47	14.15	Silage.
Do.....	8 0	1 8	4 to 8	79.75	20.25	7.13	2.13	28.89	51.16	10.69	Original.
Do.....	8 0	1 8	4 to 8	83.00	17.00	12.12	3.68	24.70	46.00	13.41	Silage.
Seeds not quite in dough stage.....	9 3	9 6	36	81.70	18.30	12.50	3.04	29.25	43.47	11.74	Original.
Do.....	9 3	9 6	36	83.50	16.50	14.62	1.65	27.57	42.46	13.70	Silage.
Do.....	7 7	2 3	4 to 8	79.00	21.00	10.25	2.61	24.95	50.50	11.69	Original.
Do.....	7 7	2 3	4 to 8	82.70	17.30	12.86	2.39	24.57	47.00	13.18	Silage.
First flower seeds well in dough stage.....	9 0	10 1	36	79.85	20.15	10.25	2.18	20.66	57.05	9.86	Original.
Do.....	9 0	10 1	36	81.72	18.28	11.56	3.88	21.46	52.37	10.73	Silage.
Do.....	10 3	6 0	4 to 8	83.00	17.00	9.76	4.04	27.60	46.63	11.97	Original.
Do.....	10 3	6 0	4 to 8	84.00	15.90	11.06	5.14	22.10	48.75	12.95	Silage.
First flower seeds well beyond dough stage.....	7 6	10 3	36	80.35	19.65	12.38	5.78	20.37	48.47	13.00	Original.
Do.....	7 6	10 3	36	80.60	19.40	13.63	5.35	23.45	42.49	15.08	Silage.
Do.....	8 10	6 0	4 to 8	81.80	18.20	11.75	1.68	24.84	40.83	11.90	Original.
Do.....	8 10	6 0	4 to 8	83.90	16.04	12.75	3.70	21.90	48.36	13.29	Silage.

## DISCUSSION OF THE COMPOSITION OF SUNFLOWERS

In studying the results on the five stages of maturity, consideration must be given to the general trend of results rather than to slight differences which may occur at the different stages of growth, for the reason that it is extremely difficult to secure uniform samples. All percentages are based on the original weight of the plant, and were found by analysis of the anhydrous material.

### DRY MATERIAL

In comparing the sunflower plants cut at different stages of maturity in the 4 to 8 inch spacing and the 36-inch spacing, there is seen very little difference in percentage of dry material, the 36-inch spacing showing only a slightly higher percentage of dry material over the 4 to 8 inch spacing. The plants in the 36-inch spacing show a more gradual increase in dry material throughout the five stages of maturity than the plants in the 4 to 8 inch spacing. The fifth stage of maturity of the sunflowers represents the stage of growth that was siloed in the large experiment station silos.

### PROTEIN

Protein is consistently higher in all the stages of maturity when the plants were spaced 36 inches apart than when they were spaced from 4 to 8 inches. The first two stages of maturity of the 36-inch spacings were considerably higher in protein than the first two stages of the 4 to 8 inch spacings. In the last three stages, the advantage of high protein remains with the 36-inch spacings, but the differences in the percentage of protein in the two spacings were not so marked as they were in the first two stages.

### CRUDE FIBER

Contrary to the general belief, it is seen that the crude fiber content of the sunflowers in both systems of plantings is not greater at the latter two stages than at the earlier stages of maturity. In fact, this table shows that the crude fiber is slightly less at the latter stage of maturity than at the earliest stage analyzed. The 36-inch spacings have less crude fiber than the 4 to 8 inch spacings. From the results it appears that the percentage of crude fiber does not increase until near the end of the growing period of the sunflower, when the seeds are matured and the stalk and leaves are dead. At this stage the percentage of moisture has decreased, the stalk has become hard and woody, and many leaves have fallen, thus increasing the crude fiber and materially lessening the feeding value of the sunflowers.

### ETHER EXTRACT

No uniformity seems to exist in the relation of the ether extract in the two different systems of spacings at the different stages of maturity. In general, the 36-inch spaced plants show a larger percentage of ether extract than the 4 to 8 inch spaced plants. Sunflowers when planted closely together produce much smaller flowers, which do not always mature, hence the ether extract is less in amount. Plants spaced far apart not only produce larger flowers but in many cases more mature ones containing a considerable quantity of seed, which in turn contain a large amount of oil, thereby increasing the percentage of ether extract.

### COMPOSITION OF LEAVES, STALKS, AND FLOWERS

The composition of leaves, stalks, and flowers was also determined. The results are given in Table II.

TABLE II.—Composition of sunflower leaves, stalks, and stems (1920)

Stage of growth.	Average height.	Average weight.	Distance apart in rows.	Moisture.	Anhydrous material.	Crude protein.	Ether extract.	Crude fiber.	Nitrogen-free extract.	Ash.	Part of the plant analyzed.
	<i>Ft. in.</i>	<i>Lbs. oz.</i>	<i>Inches.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	
Budding.....	.....	2 8	36	77.20	22.80	26.20	2.72	8.33	42.85	22.80	Leaves.
Do.....	.....	0 14	4 to 8	77.20	22.80	22.38	3.60	9.88	44.57	22.80	Do.
Do.....	8 2	8 4	36	85.60	14.40	8.56	1.38	27.38	52.13	10.55	Stalk and stems.
Do.....	9 8	3 ½	4 to 8	82.20	17.80	6.88	0.83	28.66	54.65	8.98	Do.
First flower in blossom.....	.....	3 0	36	77.70	22.30	23.57	2.94	9.06	44.16	20.27	Leaves.
Do.....	.....	1 1½	4 to 8	75.90	24.10	21.69	3.78	11.04	48.40	19.09	Do.
Do.....	.....	0 15	36	81.20	18.80	16.69	4.71	11.16	58.67	8.77	Flowers.
Do.....	.....	0 10½	4 to 8	80.80	19.20	15.81	4.57	4.87	66.50	8.25	Do.
Do.....	11 2	8 1	36	80.30	19.70	5.31	0.63	21.85	63.80	8.41	Stalk and stems.
Do.....	10 2	4 0	4 to 8	80.40	19.60	5.74	0.56	31.84	52.10	9.76	Do.
Seeds not quite in the dough stage.....	.....	1 7	36	76.40	23.60	21.94	2.05	9.87	45.06	21.08	Leaves.
Do.....	.....	1 5	4 to 8	78.20	21.80	24.19	2.97	10.91	41.71	20.22	Do.
Do.....	.....	1 0	36	83.90	16.10	15.62	5.66	10.28	60.12	8.32	Flower.
Do.....	.....	0 15	4 to 8	84.70	15.30	14.81	5.03	12.88	58.83	8.45	Do.
Do.....	8 4	5 0	36	81.00	19.00	5.31	0.51	27.98	57.17	9.03	Stalk and stems.
Do.....	10 9	4 10	4 to 8	84.20	15.80	6.12	0.49	36.94	46.37	10.08	Do.
First flower seeds well into the dough stage.....	.....	0 15	36	76.80	23.20	20.80	3.44	8.98	46.75	20.03	Leaves.
Do.....	.....	0 12	4 to 8	75.60	24.40	22.50	4.05	9.71	43.70	20.00	Do.
Do.....	.....	1 14	36	84.00	16.00	15.56	9.48	20.40	46.56	8.00	Flower.
Do.....	.....	0 14	4 to 8	82.00	18.00	13.87	5.38	11.92	61.23	7.60	Do.
Do.....	8 4	4 5	36	85.70	14.30	5.19	0.86	31.74	50.72	11.49	Stalk and stems.
Do.....	10 0	3 12½	4 to 8	80.40	19.60	5.25	1.76	32.58	52.30	8.11	Do.
First flower seeds well beyond the dough stage.....	.....	2 8	36	77.30	22.70	24.25	4.05	8.78	42.88	20.04	Leaves.
Do.....	.....	0 10½	4 to 8	75.00	25.00	20.19	4.16	11.06	42.43	22.16	Do.
Do.....	.....	2 14	36	82.30	17.70	16.12	6.27	17.44	53.27	6.90	Flower.
Do.....	.....	0 14½	4 to 8	82.80	17.20	14.31	5.94	15.60	56.28	7.87	Do.
Do.....	8 6	9 2	36	83.50	16.50	5.56	0.72	32.24	48.74	11.84	Stalk and stems.
Do.....	8 2	2 4	4 to 8	78.50	21.50	4.25	0.88	29.21	57.11	8.55	Do.

Table II is presented with a view of showing the distribution of the food nutrients in the leaves, flowers, and stalks. The table is largely self-explanatory, but a few significant facts will be discussed. The largest protein percentage content of the plant is found in the leaves. The flowers rank second and the stalks contain the smallest proportion.

When the different stages of maturity are compared in the two systems of spacing, it is seen that the percentage of protein in the leaves is quite constant throughout the various stages of growth. The 36-inch spaced plants showed a slightly higher percentage of protein in the leaves at the final stage of maturity as compared with the plants of 4 to 8-inch spacing. The percentage of protein found in the stalks and stems is the reverse of that found in the leaves when the two spacings are compared, while the percentage of protein in the flowers of both systems of plants is quite uniform. In the percentage of ether extract the flowers rank first, the leaves second, and the stalks the last in amount. The percentage of crude fiber is greatest in the stalks and stems and least in the leaves. The flowers contain an amount intermediate between the leaves and stalks, and as the flowers mature they show an increase in amount of crude fiber.

TABLE III.—Percentage of leaves, stalks, and flowers (1920)

Stage of growth.	Height.	Average weight of plants.	Distance apart in rows.	Flower.	Leaves.	Stalk and stems.
	<i>Ft. in.</i>	<i>Lbs. oz.</i>	<i>Inches.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Budding.....	8 3	10 12	36	.....	23.1	76.9
Do.....	9 8	3 15	4 to 8	.....	22.2	77.8
1st flower in blossom.....	11 2	12 0	36	7.8	25.0	67.2
Do.....	10 2	5 12	4 to 8	11.4	18.0	70.6
Seeds not quite in the dough stage.....	8 4	7 7	36	13.5	19.3	67.2
Do.....	10 9	6 14	4 to 8	13.6	19.1	67.3
1st flower seeds well into dough stage.....	8 4	7 2	36	26.3	13.0	60.7
Do.....	10 0	5 1½	4 to 8	17.7	14.7	67.6
1st flower seeds well beyond the dough stage.....	8 6	14 8	36	19.0	17.2	63.8
Do.....	8 2	3 13	4 to 8	23.8	14.0	62.2

#### SUNFLOWER PLANT PERCENTAGE OF LEAVES, STALKS, AND FLOWERS

Table III is inserted to show the percentage of leaves, stalks, and flowers. Since the analyses of these different parts showed such widely different amounts of food nutrients, this table was introduced to show whether the two systems of plantings resulted in a different percentage of leaves, stalks, and flowers. A study of Table III indicates that under these two systems of plantings no consistent variations were noted in the ratios of leaves, stalks, and flowers. The percentage of leaves and stalks decreased throughout the growing period, while the percentage of flowers increased; this was true in both systems of plantings. The actual percentage of flowers, leaves, and stalks remain quite uniform throughout the various stages of maturity in sunflower plants which are grown at the two extreme distances in the row—namely, 4 to 8 and 36 inches.

TABLE IV.—Acidity of sunflower silage (1920)

Stage of growth.	Distance apart in rows.	Moisture.	Acids in 100 gm. silage juice.		Total acids.
			Nonvola- tile acids.	Volatile acids.	
	<i>Inches.</i>	<i>Per cent.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
Budding.....	36	86. 0	2. 024	0. 382	2. 406
	4 to 8	86. 35	2. 616	. 558	3. 174
First flower in blossom.....	36	85. 0	2. 200	. 566	2. 766
Do.....	4 to 8	83. 0	2. 640	. 653	3. 293
Seeds not quite in dough stage....	36	83. 5	1. 896	. 509	2. 405
Do.....	4 to 8	82. 7	2. 740	. 600	3. 340
First flower seeds well in dough stage	36	81. 7	2. 648	. 728	3. 376
Do.....	4 to 8	84. 1	2. 376	. 613	2. 989
First flower seeds well beyond dough stage.....	36	80. 6	2. 536	. 700	3. 236
Do.....	4 to 8	83. 9	3. 000	. 623	3. 623

Stage of growth.	Dis- tance apart in rows.	Mois- ture.	Acids in 100 gm. wet silage.		Total acids.	Acids in 100 gm. anhydrous silage.		Total acids.
			Non- vola- tile.	Vola- tile.		Non- vola- tile.	Vola- tile.	
	<i>Inches.</i>	<i>Per cent.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
Budding.....	36	86. 0	1. 741	0. 329	2. 070	10. 69	2. 020	12. 71
	4 to 8	86. 35	2. 260	. 482	2. 742	14. 30	3. 049	17. 35
First flower in blossom.....	36	85. 0	1. 870	. 481	2. 351	10. 68	2. 747	13. 43
Do.....	4 to 8	83. 0	2. 191	. 542	2. 733	10. 70	2. 646	13. 35
Seeds not quite in dough stage.....	36	83. 5	1. 585	. 425	2. 010	8. 09	2. 170	10. 26
Do.....	4 to 8	82. 7	2. 267	. 496	2. 763	10. 84	2. 371	13. 21
First flower seeds well in dough stage.	36	81. 7	2. 162	. 595	2. 757	9. 71	2. 657	12. 37
Do.....	4 to 8	84. 1	2. 000	. 516	2. 516	10. 59	2. 730	13. 32
First flower seeds well beyond dough stage	36	80. 6	2. 045	. 564	2. 609	8. 53	2. 350	10. 88
Do.....	4 to 8	83. 9	2. 518	. 523	3. 041	13. 10	2. 723	15. 82

## ACIDITY OF SUNFLOWER SILAGE

Table IV shows that there is more acidity formed when sunflowers are siloed in the later stages of growth than when siloed in the earlier stage. This fact is different in corn silage, since immature corn always produces more acidity than corn siloed at a mature stage. The total amounts of acidity, however, found in these samples of sunflower silage easily fall within the amounts usually found in good sunflower silage. It must be remembered that kind of acids present is more of a criterion of good silage than quantity of acids. All silage samples made at the different stages of growth were classed as good samples when acidity, odor, and color were used as the criterion. The earlier stages would not be as suitable for silage purposes as the later stages, due to the large percentage of moisture which they contained. Even the last stage cut contained slightly higher than 80 per cent of moisture, but it must be remembered that these samples were not allowed to be exposed to the

sun after cutting, hence they represent the amount of moisture present immediately at the time of cutting. In the general farm practice of siloing sunflowers there is usually a loss of a few per cent of moisture by the slight wilting of the plants which takes place during the time of cutting and hauling to the silage cutter. The plants cut at this last stage of maturity were siloed in the large experiment station silos and produced excellent silage.

#### COMPOSITION OF SUNFLOWERS IN 1921

Additional work was carried on in 1921 on sunflowers planted singly and in hills at different distances apart in the row. The different distances apart in the rows selected were 8, 24, and 42 inches. In the hill system of planting there were one, two, three, and four stalks to the hill. All hills were planted 42 inches apart in the row. In both the drilled and hill systems the rows were 42 inches apart, this being the width of the planter.

The results of the analyses of the five stages of plants are given in Table V. All results except acre yield are given on the anhydrous basis.

In comparing the different systems of planting, 8, 24, and 42 inches apart in the row, and the hill systems, one, two, three, and four plants in the hill, it is noticeable that the 8-inch spacings produced the highest yield of all. This system is closely followed by the hill system of planting where three stalks were allowed to grow in each hill.

#### ANHYDROUS MATERIAL

The same conclusions as those found in 1920 are drawn when the amount of anhydrous material is considered. The 8-inch spacing is highest in amount with the three stalks in a hill system closely following. Very little distance is noted in any of the other systems of planting.

#### CRUDE PROTEIN

Crude protein gradually decreases as the sunflowers become more mature. The amount of protein is quite similar for all systems of plantings, averaging between 9 and 10 per cent in the final stages, the only exception to this being the four stalks in a hill system, which shows only 7.98 per cent crude protein in the final stage analyzed.

#### CRUDE FIBER

It is a noticeable fact that crude fiber increases up to the fourth stage, then decreases somewhat in the final stage examined. This fact holds true in both 1920 and 1921, with the exception of the 8-inch spacings grown in 1921.

#### ETHER EXTRACT

Ether extract is considerably higher in the last two than in the first three stages analyzed.

TABLE V.—Composition of sunflowers (1921)

Stage of growth.	Height. Ft. in.	Weight. Lbs. oz.	Distance apart in rows.	Acre tons. <sup>a</sup>	Number in hill.	Moisture. Per cent.	Anhy- drous material. Per cent.	Crude protein. Per cent.	Ether extract. Per cent.	Crude fiber. Per cent.	Nitrogen free extract. Per cent.	Ash. Per cent.
Budding.....	6 6	1 5 1/2	8	.....	1	81.4	18.6	10.38	1.82	26.40	48.72	12.68
First flower in blossom.....	6 1	1 8 1/2	8	.....	1	85.44	16.56	8.44	2.29	25.50	52.27	11.50
Seeds not quite in dough stage.....	6 1	1 14	8	.....	1	82.05	17.95	8.86	2.26	27.30	48.89	12.75
First flower seeds well into dough stage.....	6 1	2 1	8	.....	1	81.4	18.6	7.94	2.55	27.10	49.66	12.75
First flower seeds well beyond dough stage.....	6 0	2 15	8	11.36	1	77.4	22.6	9.32	10.02	28.15	41.76	10.75
Budding.....	6 0	2 6 1/2	24	.....	1	82.4	17.6	11.60	1.72	26.10	46.53	14.05
First flower in blossom.....	6 0	2 13	24	.....	1	81.93	18.07	10.40	2.26	27.10	48.39	11.85
Seeds not quite in dough stage.....	6 4	3 8	24	.....	1	82.2	17.8	9.72	1.92	30.30	46.91	11.15
First flower seeds well into dough stage.....	6 6	4 5	24	.....	1	82.53	17.47	9.32	1.93	31.50	46.13	11.15
First flower seeds well beyond dough stage.....	6 0	4 14	24	7.72	1	79.4	20.6	10.90	1.63	29.10	41.83	10.79
Budding.....	5 10	5 3 1/2	42	.....	1	84.14	15.86	13.90	1.70	27.50	43.30	14.97
First flower in blossom.....	5 5	5 2 1/2	42	.....	1	85.53	14.47	12.80	1.76	27.50	44.63	13.37
Seeds not quite in dough stage.....	5 11	6 2	42	.....	1	83.57	16.43	11.35	2.25	25.90	49.39	11.60
First flower seeds well into dough stage.....	5 11	8 0	42	.....	1	83.8	16.2	11.98	2.57	31.70	42.40	11.67
First flower seeds well beyond dough stage.....	5 6	8 3	42	9.72	1	79.4	20.6	9.53	5.87	27.75	40.95	9.90
Budding.....	5 11	2 8 1/2	42	.....	2	83.4	16.6	10.45	1.57	29.80	45.18	13.00
First flower in blossom.....	6 2	2 12	42	.....	2	83.67	16.33	10.55	1.97	27.80	46.11	13.47
Seeds not quite in dough stage.....	6 2	3 6 1/2	42	.....	2	82.23	17.77	10.90	2.07	27.75	46.28	13.00
First flower seeds well into dough stage.....	5 10	4 5 1/2	42	.....	2	82.07	17.93	10.71	3.75	30.15	43.24	12.15
First flower seeds well beyond dough stage.....	6 0	5 1/2	42	10.28	2	79.2	20.8	9.75	5.56	27.46	47.98	9.25
Budding.....	6 4	1 15	42	.....	3	82.9	17.1	10.06	1.47	25.70	49.62	13.15
First flower in blossom.....	6 2	2 1	42	.....	3	81.87	18.13	9.35	2.11	25.70	51.39	11.45
Seeds not quite in dough stage.....	6 5	2 14	42	.....	3	82.43	17.57	10.00	1.75	31.30	44.30	12.65
First flower seeds well into dough stage.....	6 4	4 1	42	.....	3	83.0	17.0	10.10	2.43	30.40	45.02	11.65
First flower seeds well beyond dough stage.....	6 0	4 6	42	10.58	3	78.14	21.86	10.22	6.61	28.45	44.62	10.30
Budding.....	6 0	2 2	42	.....	3	84.54	15.46	10.25	1.32	23.80	52.38	12.35
First flower in blossom.....	6 1	2 1/2	42	.....	4	82.4	17.6	10.30	1.90	27.04	49.01	11.75
Seeds not quite in dough stage.....	6 5	2 11 1/2	42	.....	4	84.25	15.75	10.85	1.61	30.40	43.89	13.25
First flower seeds well into dough stage.....	5 10	2 12	42	.....	4	79.7	20.3	10.33	3.06	29.70	46.31	11.60
First flower seeds well beyond dough stage.....	6 3	2 11	42	9.58	4	78.6	21.4	7.98	6.32	26.10	48.70	10.90

<sup>a</sup> Acre yields were furnished by the agronomy department of the University of Idaho.



## COMPARISON OF YIELDS, 1920 AND 1921

The yield per acre of sunflowers grown in 1921 was slightly less than half the yield secured in 1920, due to dry weather. The total precipitation for each month during the growing season is given in the following table:

TABLE VI.—*Precipitation during the growing season*

Month.	1920.	1921.	Month.	1920.	1921.
	<i>Inches.</i>	<i>Inches.</i>		<i>Inches.</i>	<i>Inches.</i>
April.....	2. 72	2. 86	July.....	0. 54	0. 19
May.....	1. 35	1. 80	August.....	1. 22	0. 30
June.....	1. 16	1. 47	September.....	2. 52	1. 43

## CONCLUSIONS

A comparison of the results on sunflowers grown in Idaho and those grown by Shaw and Walters at Beltsville, Md., shows some striking facts. Probably, due to climatic conditions, Shaw and Wright were able to secure a more advanced stage of maturity of their sunflower crop as the last stage represented the production of good hard seed. As has been stated before, it is seldom possible to secure such mature sunflowers in certain portions of the Northwest. The crop grown for this experimental work is a good example, since the last stage analyzed represents the maximum growth. At this period a frost occurred and the sunflowers were siloed.

In content of dry material Idaho sunflowers when siloed represent Shaw and Wright sunflowers matured in stages between rays partly fallen and rays all fallen. It appears from these results, and results of previous years, that the crude protein is considerably higher in sunflowers grown in the Palouse country of Idaho than in Maryland, one crop grown in 1920 representing 12.38 per cent, and one in 1921 10 per cent, as against approximately 7 per cent in Maryland for the same stage of maturity. The sunflowers grown in Montana, analyzed by Blish, show lower percentages of protein than sunflowers grown in the Palouse section of Idaho. From these results it would appear unfair to compare sunflowers grown in different parts of the United States for relative food value, since the composition of sunflowers may vary widely, owing to the differences in climatic and soil conditions.

The 1920 results on the composition of sunflowers at different periods of growth show that the crude fiber does not continue to increase as the plant passes through the various stages of maturity. Similar results were obtained with the 1921 crop. The percentage of crude fiber found in the sunflowers cut at the last stage of growth was slightly less than in the more immature stages studied. From these data, it appears that there is practically no gain in crude fiber during the growth of the sunflower plant throughout the five stages of maturity included in this investigation. The crude fiber may increase materially if the sunflowers are allowed to mature completely. At this time the leaves are dead and partly fallen and the stalk becomes hard and woody. Silage made from sunflowers in this stage of maturity is high in crude fiber and is not palatable. It is possible that some of the unfavorable results which have been noted in isolated cases with sunflowers have been due to the fact that sunflowers were allowed to mature too far before they were cut for silage.

## TIME FOR CUTTING

In considering the proper stage for cutting sunflowers in Idaho, either of the last two stages could be profitably used for silage, as there is little material difference in the analyses for the two stages in either the 1920 or 1921 crops. In some sections where sunflowers mature slightly more than in the Palouse country of Idaho, it might be profitable to cut them a little later than those of the last stage included in this investigation. In any event, cutting should not be delayed much beyond the last stage studied, for then the stalks become hard and woody and a silage is obtained which is unpalatable and not relished by the stock.

The moisture content of the sunflower plant grown in the last two stages is approximately 80 per cent. This percentage of moisture is slightly higher than is usually desired in a crop intended for the silo. When the green material contains too much moisture, there is danger of a loss of considerable juice, which means a loss of soluble food nutrients. In crops containing a high sugar content, a high moisture content means also a high acid content. Shaw and Wright state that "a high moisture in the plant is usually associated with high acid silage." This holds true for corn silage, but an inspection of Table I shows that it does not always hold true for sunflowers. In the case of sunflowers a high moisture content results chiefly in a loss of soluble food nutrients in the juice.

If the sunflowers seem to have too high a moisture content when cut, this can be materially decreased by allowing the plant to wilt for a short period. Blish, of Montana, has shown that excellent silage resulted when sunflowers were cut and allowed to wilt before siloing until the moisture content was 72.24 per cent. A moisture content for sunflower silage in Idaho can vary from 70 to 80 per cent, the writers recommending approximately 75 per cent as an average.

## DISTANCE APART IN ROW

When the two different distances of planting in the row are considered for 1920, it appears that the single plants growing 36 inches apart in the row rank slightly higher in food nutrients than the sunflowers planted every 4 to 8 inches. The analyses of the sunflowers, and the sunflower silage, both show a slight advantage over the closer plantings. The yield must be considered in deciding which system of planting is most profitable. The yields for the two spacings determined by the agronomy department showed 22.32 tons for the 36-inch spacings and 20.23 tons for the 4-inch to 8-inch spacings. However, because of the difference in the contour of the land upon which the sunflowers were grown, these yields can not be considered as final.

The yields of sunflowers grown in 1921 show results which are contrary to the findings of 1920. The 8-inch spacings gave a higher yield than the 42-inch spacings. Under the conditions that existed in 1921, very little difference was noted in the yields of the sunflowers drilled 8 inches apart and the sunflowers planted in hills containing two or three stalks. Four stalks in the hill did not yield as well as the hills containing two and three stalks.

From the data presented on the two years of sunflowers it is apparent that the different climatic conditions, chiefly the proper distribution of moisture throughout the life cycle of the sunflower plant, affect the yield of sunflowers when planted at different spacings in the row. The results vary from year to year in the Palouse country, because of variable

climatic conditions, to such an extent that no specific recommendations can be made as to the best systems of planting. Where irrigation is practiced, and the moisture supply controlled, more regular yields would be expected under the different systems of planting. It is hoped that these studies on sunflowers grown under irrigation will be continued. The results in this paper are representative of what may be expected when sunflowers are grown in sections of the United States where climatic conditions and altitude are similar to those in the Palouse country. The choice of the system of planting sunflowers, grown without irrigation, must necessarily rest with the farmer, depending upon whether it is more feasible to harvest and silo the large type of sunflowers rather than the smaller type. In choosing the system of planting, the farmer should consider the difference in labor involved in harvesting the large or small type of sunflowers.

# EFFECT OF DIFFERENT CONCENTRATIONS OF MANGANESE SULPHATE ON THE GROWTH OF PLANTS IN ACID AND NEUTRAL SOILS AND THE NECESSITY OF MANGANESE AS A PLANT NUTRIENT<sup>1</sup>

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## INTRODUCTION

In a study of the literature on the nutrition of plants, one is impressed with the lack of definite knowledge concerning the necessity of a number of elements frequently found in appreciable amounts in the ash of different species.

For many years it has been taught and accepted by agronomists and plant physiologists that only 10 of the elements are essential for the normal growth and maturation of plants. These essential elements are carbon, hydrogen, oxygen, nitrogen, calcium, magnesium, potassium, phosphorus, sulphur and iron. In addition to these, it is also well known that as many as 20 or 30 others are frequently found in small amounts in plants which have grown under natural conditions.

Within recent years many data have been obtained which indicate that a few of these so-called nonessential elements may have important functions in the plant's economy. During the past 25 years, perhaps, more attention has been given to the acquisition of knowledge concerning the occurrence, distribution, and probable functions of manganese in soils and plants than to any other one of the elements referred to.

The number of contributions to the literature on the relation of manganese to various phases of agriculture number no less than 100. As a matter of convenience these contributions may be divided into two general classes, depending upon the object to be attained. Investigators of one class, apparently, have been interested in determining whether or not manganese has any commercial value from the standpoint of a necessary fertilizer, while those of the other class have sought to determine whether or not manganese is an essential element in the vegetable economy and, if so, to ascertain its functions.

## HISTORICAL

Manganese was discovered by Scheele (9)<sup>2</sup> in 1774. He found that the soil contained small amounts of this element and that it was assimilated by plants that grew in the soil; however, he made no effort to determine whether manganese was necessary for plant growth.

In 1864 Sachs (8) conducted experiments, the purpose of which was to determine whether or not manganese was necessary in plant economy. He proved that manganese could not replace iron in plant growth, but

<sup>1</sup> Accepted for publication Nov. 6, 1922.

This investigation, begun at the Kentucky Agricultural Experiment Station in 1916, was continued, with the consent of the Director, at Cornell University where part of the results were offered in partial fulfillment of the requirements for the degree of doctor of philosophy. Since September, 1922, the work has been carried on at the Kentucky Station. The paper presents in part the results of the investigation at Cornell, the earlier results, and the recent findings at the Kentucky Station.

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 793-794.

he did not prove that manganese was also necessary when iron was available.

In 1883 Yoshida (12) isolated from the sap of the lac tree a product possessing peculiar enzymic properties which he was unable to explain.

In 1894 Bertrand (1) undertook further work on the nature and composition of the product which had been isolated from the sap of the lac tree, and found that it contained considerable manganese, which gave to the product its peculiar enzymic properties. After several years of research on the relation of manganese to the growth of plants he concluded that this element is necessary for the normal growth of both autotrophic and heterotrophic plants and that its functions can not be performed by any other element.

In 1914 Brenchley (2) published the results of an investigation concerning the effect of manganese sulphate on the growth of barley seedlings. She found that with a concentration of  $1/100,000$  of  $MnSO_4$  and less a decided stimulation in the growth was produced; with greater concentrations than this, however, toxic effect resulted. In her conclusion with respect to the function of manganese in plants she suggests the probability that manganese may prove to be an essential element in the economy of plant life, even though the quantity usually found in plants is very small.

Kelly (5) made a study of the effect produced on different species of plants when grown in a natural soil containing a large amount of manganese as compared with the effect produced on the same species when grown on a soil containing only a normal amount of manganese. From his results he concluded that the small amount of manganese occurring in soils probably performs a twofold function in plant growth; (1) It acts catalytically, increasing the oxidations in the soil and accelerating the auto-oxidations in plants; and (2) it tends to modify the absorption of calcium and magnesium, perhaps by partially replacing calcium from insoluble combinations, but especially through a direct effect by which the absorption of calcium is increased and that of magnesium is decreased.

McHargue (6) grew wheat plants to maturity in solution cultures free from and containing manganese and found that in the absence of manganese the plants became etiolated and did not develop in a normal way, thus showing that manganese is essential to the normal growth and maturation of this plant.

Previous to 1902 but little attention had been given to the occurrence and distribution of manganese in soils. During that year Ewell (3) made an interesting observation with respect to the occurrence of soluble salts of manganese in a certain small area of soil which failed to produce crops in a normal way. The results of his investigation showed that the small nonfertile area of soil contained relatively large amounts of soluble salts of manganese, while the adjacent fertile soil contained none. He assumed that the difference in the fertility of the two soils was due to the presence of the soluble compounds of manganese. The publication of his conclusions apparently awakened a wide interest regarding the rôle manganese plays in soils.

In 1907 the United States Bureau of Soils (10) inaugurated a series of experiments with manganese at the Arlington Experiment Farm. Manganese sulphate was applied at the rate of 50 pounds per acre to an acid silty clay loam soil, and crops were grown on the treated and untreated soil for six years. The application of manganese to the acid soil resulted

in decreased yields; however, upon neutralizing the acidity in the soil by the addition of calcium carbonate the plots receiving manganese produced very marked increases in yields, thus showing that the reaction of the soil is an important factor in determining whether or not an application of manganese sulphate will exert a beneficial effect on plant growth.

Funchess (4) has obtained data which tend to show that some acid soils contain soluble salts of manganese. He also offers the suggestion that the toxic effect associated with soil acidity may be due in part to soluble salts of this element. He describes experiments with acid soils that contained soluble salts of manganese and were toxic to the growth of plants. He found that the toxic effect of the soil was destroyed after neutralization with calcium hydroxid.

In 1920 Olaru (7) published results showing the effect of manganese on some of the more important microorganisms concerned with nitrogen transformations in soils and leguminous plants. With pure cultures of *Bacillus radiocicola*, *Azotobacter chroococcum*, *Clostridium pasteurianum*, and *Micrococcus ureae* and with concentrations of manganese sulphate varying from zero to 10 mgm. per liter he obtained a marked stimulation in the functions of each of these organisms. The maximum stimulation appears to have been obtained with concentrations of about 1 mgm. of manganese per liter. He therefore concludes that the small amount of manganese occurring in the soil serves a very useful function with respect to the organisms concerned in nitrogen transformation.

Robinson (11) has determined the manganese content of 26 different and representative soil types in this country. The maximum amount found in any soil was 0.51 per cent, the minimum 0.01 per cent, and the average 0.20 per cent of MnO, respectively. Various other reports concerning the amount of manganese contained in the soils of this and other countries will average approximately 0.10 per cent of this element, which is as much as the phosphorus and sulphur contained in soils that produce average yields of field crops.

In the foregoing review it has been the aim of the author to refer to those contributions on the subject which contain the more modern views concerning the probable function of manganese in its relation to agriculture.

The purpose of this investigation was, first, to show the effect of increased concentrations of manganese sulphate on the growth of plants in certain soils, and, secondly, to determine definitely if manganese is an essential element in the plant economy.

#### EXPERIMENTAL DATA

Citation (4) is a contribution whose data indicate that soluble salts of manganese are associated with and may in part be responsible for the toxicity of acid soils. To obtain further data on this subject, a series of experiments was planned in which plants were grown in acid and in neutralized portions of the same soil to which were added equal and graduated amounts of manganese sulphate. The soils selected for the experiments were not only acid but were lacking in capacity to produce good crops without the addition of certain plant nutrients.

## EXPERIMENTS WITH VOLUSIA SILT LOAM SOIL

Volusia silt loam soil has a brownish-gray color, is low in organic matter, and contains considerable clay. It requires about 2,000 pounds of calcium carbonate per acre foot to neutralize its acidity.

About 500 pounds of this soil were obtained in a pasture field on Turkey Hill, Tompkins County, N. Y. The soil was well mixed and allowed to air-dry. Representative samples were taken for determination of the total and water-soluble manganese.

The total manganese content was determined by the potassium bisulphate fusion and the colorimetric periodate method. The amount of manganese found by this method was 0.08 per cent, or 800 parts per million of the air-dry soil. The amount of manganese dissolved by digestion with distilled water was 6.25 parts per million of the air-dry soil.

For pot experiments, 4,000 gm. portions of the air-dry soil were weighed into clean 1-gallon earthenware jars without drainage. Two series of 12 jars each were prepared, one with and the other without calcium carbonate, as shown in Table I, each treatment being made in duplicate. The mineral nutrients consisted of 10 gm. calcium nitrate, 10 gm. dipotassium phosphate, and 5 gm. magnesium sulphate per jar.

TABLE I.—*Pot treatments in Series I and II*

Pot No.	Treatment, Series I.	Treatment, Series II.
1 and 1a...	No treatment.....	20 gm. CaCO <sub>3</sub> .
2 and 2a...	Mineral nutrients only.....	Mineral nutrients and 20 gm. CaCO <sub>3</sub> .
3 and 3a...	Mineral nutrients and 5 parts per million Mn.	Mineral nutrients, 20 gm. CaCO <sub>3</sub> and 5 parts per million Mn.
4 and 4a...	Mineral nutrients and 10 parts per million Mn.	Mineral nutrients, 20 gm. CaCO <sub>3</sub> and 10 parts per million Mn.
5 and 5a...	Mineral nutrients and 50 parts per million Mn.	Mineral nutrients, 20 gm. CaCO <sub>3</sub> and 50 parts per million Mn.
6 and 6a...	Mineral nutrients and 100 parts per million Mn.	Mineral nutrients, 20 gm. CaCO <sub>3</sub> and 100 parts per million Mn.

Purple-top radish seeds were sown in each pot and the moisture content of the soil in each pot was brought to one-half saturation, by weight, with distilled water. After the seedlings were up they were thinned to 10 plants in each pot and the moisture content kept at approximately one-half saturation with distilled water during the time the plants were making their growth.

Immediately after the crop of radishes was harvested soybeans were planted in each of the pots without further treatment of the soil. After the beans had reached the proper size they were thinned to 6 plants in each pot and allowed to grow until they were approaching maturity, the moisture content being meanwhile kept up to about one-half saturation with distilled water.

Plate 1, A, represents five pots from series No. 1. Pot No. 1 is one of the control pots and represents the productivity of the soil without treatment. Pot No. 2 shows the effect of the addition of appropriate amounts of phosphorus, potassium, calcium, magnesium, nitrogen and sulphur to the soil. This is one of the pots that received mineral nutrients

only and no manganese. Pot No. 3 received 5 parts per million of manganese in addition to the mineral nutrients. Pot No. 4 received 10 parts per million of manganese. Pot No. 5 received 50 parts per million of manganese. It is therefore readily apparent that 50 parts per million of manganese in the form of manganese sulphate are sufficient to produce toxicity in this soil. None of the seeds came up in the soil receiving this and greater concentrations of manganese sulphate. It is quite evident from Plate 1, A, that the maximum tolerance for manganese sulphate in this soil and with this particular plant lies between 10 and 50 parts per million.

Table II shows that the plants in the corresponding duplicate pots which are not shown made a similar growth.

There is no noticeable difference in the growth of the plants receiving the 5 and 10 parts per million of manganese, as compared with those that received no additional manganese. It is apparent that the small amount of manganese contained in the soil was sufficient for the requirements of the plants, under the conditions described.

The growth attained by the plants in the neutralized Volusia soil is shown in Plate 1, B, in which pot No. 1 is one of the control pots to which calcium carbonate was added. Comparison with the corresponding pot in Plate 1, A, shows that the plants made a slightly better growth in the neutralized soil than in the acid soil. Pot No. 2 received mineral nutrients and calcium carbonate and the plants did not make as good growth as did those in the corresponding pots with the acid soil. Pot No. 4 is one of two to which 10 parts per million of manganese was added. Neither of the two pots of soil that received 5 parts per million of manganese is shown in the photograph, but it will be seen in Table II that the plants that received 5 parts per million of manganese in series No. 2 made a slightly better growth than did the plants in the corresponding pots of soil in series No. 1. It will also be observed in Table II that the plants in the pots of soil that received 10 parts per million of manganese made a decidedly better growth in the neutralized soil than did the plants in the corresponding pots in the acid soil. Pot No. 5 shows the growth the plants attained in the neutralized soil to which 50 parts per million of manganese was added. It will be recalled that in the corresponding pots in the acid soil, the seeds did not germinate because of the toxic effect produced by an excess of manganese, while with the same concentration of manganese in the neutral soil the plants made a fair growth, though apparently retarded by an excess of this element. This experiment illustrates in a very striking way the effect of calcium carbonate in reducing the toxicity produced by an excess of manganese sulphate. In pot No. 6, to which was added 100 parts per million of manganese, no plants grew.

In Table II it will be observed that the applications of manganese to the acid soil reduced the yields, as shown by the green and dry weights of the plants, whereas with the same concentrations of manganese in the neutralized soil there was an appreciable gain.



TABLE II.—Weights of radish and soybean plants grown in Volusia and Dunkirk soils, with and without nutrients,  $MnSO_4$  and  $CaCO_3$ , duplicate pots

Crop.	Soil.	Acid reaction of soil.				Neutral reaction of soil.				
		No treatment.	Minerals.	Minerals plus 5 parts per million Mn.	Minerals plus 10 parts per million Mn.	$CaCO_3$ .	$CaCO_3$ plus minerals.	$CaCO_3$ plus minerals and 5 parts per million Mn.	$CaCO_3$ plus minerals and 10 parts per million Mn.	$CaCO_3$ plus minerals and 50 parts per million Mn.
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
Green weight of radish tops and roots.	{ Volusia....	45.0	212.5	167.5	152.5	52.5	151.0	178.5	194.0	36.5
	{ Dunkirk...	92.5	240.5	205.0	101.0	95.0	129.0	185.0	222.5	146.5
Dry weight of radish tops and roots.	{ Volusia....	3.3	12.4	10.0	9.7	3.4	10.7	12.9	13.4	4.2
	{ Dunkirk...	7.3	15.8	13.2	8.8	7.8	7.7	12.3	13.8	10.1
Green weight of soybeans.	{ Volusia....	38.8	65.5	50.0	28.0	42.0	42.0	60.5	45.5	11.8
	{ Dunkirk...	30.3	67.8	41.3	20.5	27.5	27.5	50.8	44.3	48.5
Dry weight of soybeans.	{ Volusia....	4.4	12.5	9.6	6.0	9.3	9.9	12.5	10.1	2.6
	{ Dunkirk...	6.9	11.9	8.2	3.6	0.2	7.1	9.5	8.8	10.0

## EXPERIMENTS WITH DUNKIRK CLAY LOAM SOIL

Two series of experiments similar to those just described with the Volusia soil were conducted at the same time and under the same conditions with the Dunkirk clay loam soil. Apparently this soil is slightly more productive than the Volusia silt loam soil. It has an acid reaction and responds to applications of certain plant nutrients.

A manganese determination on the Dunkirk soil gave 0.093 per cent of this element. The amount of water-soluble manganese found was 9.30 parts per million of the soil.

The radish plants which were grown on the Dunkirk soil under the same conditions as those previously described on the Volusia soil were harvested on the same day and treated in like manner for the green and dry weights, which are given in Table II.

No photographs were made of the plants grown in the Dunkirk soil; however, it will be seen from the green and dry weights of the plants, shown in Table II, that results somewhat comparable to those obtained with the Volusia soil were obtained under similar treatments with this soil. In series No. 1 the maximum yields occurred with the applications of mineral nutrients. Addition of manganese gave a diminution in yields as compared with the pots receiving mineral nutrients alone.

In series No. 2, application of calcium carbonate to the soil of pots No. 1 and 2 affected the growth of the plants but little as compared with the corresponding pots in series No. 1, in which the plants grew in acid soil. It thus appears that the application of calcium carbonate under these conditions had no effect as far as the growth of the plants was concerned. There is an increase which is apparently due to the applications of manganese in this series, as compared with the pots receiving mineral nutrients alone. This is in accord with the results obtained with applications of manganese to the neutralized Volusia soil.

Equal quantities of the radish tops grown in duplicate pots were combined to make a composite sample for chemical analysis, the results of which are given in Table III.

TABLE III.—Analyses of composite samples of radish tops grown in Volusia and Dunkirk soils, with and without the addition of nutrients, manganese sulphate, and calcium carbonate (results calculated on moisture-free basis)

Acid reaction of soil.						Neutral reaction of soil.				
Element.	Soil.	No treatment.	Minerals	Minerals plus 5 parts per million Mn.	Minerals plus 10 parts per million Mn.	CaCO <sub>3</sub>	CaCO <sub>3</sub> plus minerals.	CaCO <sub>3</sub> plus minerals and 5 parts per million Mn.	CaCO <sub>3</sub> plus minerals and 10 parts per million Mn.	CaCO <sub>3</sub> plus minerals and 50 parts per million Mn.
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Ash.....	Volusia....	21.89	25.60	25.51	24.45	22.06	24.96	25.49	26.00	21.71
	Dunkirk...	20.15	24.83	24.58	26.76	17.55	19.06	25.08	25.56	25.45
	Volusia....	.077	.059	.050	.091	.047	.095	.096	.071	.08
Fe .....	Dunkirk...	.073	.073	.073	.073	.074	.084	.095	.091	.11
	Volusia....	.046	.035	.058	.94	.054	.046	.070	.087	1.15
Mn.....	Dunkirk...	.024	.024	.056	.67	.019	.021	.034	.059	.84
	Volusia....	3.86	3.59	3.12	3.25	3.01	4.39	4.44	4.54	2.81
Ca.....	Dunkirk...	3.10	3.32	3.23	3.95	2.72	2.85	2.91	2.96	2.98
	Volusia....	.46	.45	.41	.38	.43	.38	.40	.44	.38
Mg .....	Dunkirk...	.43	.40	.42	.43	.45	.42	.41	.42	.34
	Volusia....	.25	.25	.78	.72	.24	.70	.70	.65	.68
P.....	Dunkirk...	.27	.56	.55	.50	.25	.59	.58	.61	.60
	Volusia....	4.56	4.72	6.67	7.47	6.94	4.33	5.55	6.52	6.35
K .....	Dunkirk...	4.56	4.56	7.69	7.99	7.31	3.52	3.16	7.83	7.24
	Volusia....	6.04	6.79	6.58	6.71	6.15	6.48	6.52	6.45	7.81
N .....	Dunkirk...	5.56	6.77	7.22	6.51	3.19	3.58	5.17	5.04	5.56

An examination of the results shown in Table III reveals some points of interest. The plants which grew in the untreated soil contained less mineral matter than did those to which mineral nutrients were added. The addition of manganese to the soil in series No. 1 did not increase the mineral content of the plants receiving it, whereas it appears to have done so in the neutralized soil, except in pots 9 and 10, in which toxicity was produced. About as much mineral matter was taken up by the plants grown in the soil to which calcium carbonate was added as where it was omitted in the corresponding control pots of soil in series No. 1. A little more calcium was taken up by the plants under neutral conditions of the Volusia soil than under acid conditions; however, a little less calcium was found in the plants that grew in the neutralized Dunkirk soil than in those that grew in the acid soil. A larger percentage of potassium was found in the plants that grew in the acid Volusia and Dunkirk soils to which manganese was added than in those plants that grew in the soil to which no manganese was added. There was an increase in the percentage of nitrogen in the plants to which manganese was added in the neutral Dunkirk soil, while the percentage of nitrogen was nearly constant in the other experiments.

It appears, therefore, that in the soils dealt with in the foregoing series of experiments with radish plants, there has been a slight gain in each of the soils where manganese was added to the neutral soil in connection with mineral nutrients. Under acid conditions there has been a diminution in the yield of the plants receiving manganese when compared with the pots receiving mineral nutrients alone. All the soils have responded vigorously to an application of mineral nutrients when compared with no treatment.

An inspection of the green and dry weights of the soybean plants in Table II indicates that a more favorable growth was obtained in the neutralized soil plus manganese than in the acid soil to which manganese

was added. The protective action of calcium carbonate against the toxic properties of an excess of manganese sulphate is demonstrated with soybeans. The plants receiving 50 parts per million of manganese in the neutral soil made a moderate growth, while in the corresponding pots of acid soil no plants were produced.

After the dry weights were determined the leaves and stems of the soybean plants in duplicate pots were combined to make a composite sample for chemical analysis. The results of the analyses are given in Table VI.

TABLE IV.—Analyses of composite samples of soybean plants grown after radish plants in Volusia and Dunkirk soils, with and without the addition of nutrients,  $MnSO_4$  and  $CaCO_3$  (results calculated on moisture-free basis)

Crop.	Soil.	Acid reaction of soil.				Neutral reaction of soil.				
		No treatment.	Minerals.	Minerals plus 5 parts per million Mn.	Minerals plus 10 parts per million Mn.	$CaCO_3$ .	$CaCO_3$ plus minerals.	$CaCO_3$ plus minerals and 5 parts per million Mn.	$CaCO_3$ plus minerals and 10 parts per million Mn.	$CaCO_3$ plus minerals and 50 parts per million Mn.
		Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
Ash.....	Volusia...	8.38	12.43	13.86	14.24	9.48	12.52	12.59	13.15	10.72
	Dunkirk...	9.58	13.17	13.98	14.12	11.36	12.50	13.19	13.86	9.37
Fe.....	Volusia...	.059	.078	.078	.069	.068	.059	.071	1.06	.114
	Dunkirk...	.070	.070	.066	.051	.050	.052	.047	.052	.082
Mn.....	Volusia...	.012	.012	.186	.303	.012	.014	.027	.042	.204
	Dunkirk...	.023	.019	.162	.277	.069	.105	.346	.697	.186
Ca.....	Volusia...	1.19	1.69	1.89	1.81	2.05	2.05	2.04	1.96	1.62
	Dunkirk...	1.90	1.87	1.60	2.12	2.08	1.62	1.88	1.84	1.71
Mg.....	Volusia...	.31	.29	.30	.28	.32	.30	.27	.28	.24
	Dunkirk...	.33	.36	.31	.29	.31	.34	.28	.25	.36
P.....	Volusia...	.13	.31	.35	.43	.18	.27	.24	.22	.36
	Dunkirk...	.23	.45	.50	.55	.27	.48	.46	.40	.41
K.....	Volusia...	1.43	3.40	3.62	3.92	1.40	3.12	3.05	3.05	3.03
	Dunkirk...	1.34	2.80	3.39	2.20	3.51	3.71	3.51	3.34	3.21
N.....	Volusia...	3.40	4.06	4.07	4.12	3.69	4.16	3.91	4.00	4.49
	Dunkirk...	1.62	4.25	3.85	3.76	1.86	3.82	3.80	3.76	3.96

Table IV shows that the smallest percentage of ash was taken up by the plants grown in the untreated soils, while the largest percentage was found in the plants that had grown in the acid soil and to which 10 parts per million of manganese was added. Considerably more manganese than iron was taken up by the plants that grew in each of the acid soils to which manganese was added. Under neutral conditions more manganese than iron was absorbed by the plants that grew in the Dunkirk soil. The results for calcium vary to some extent between the two soils but apparently more calcium was taken up by the plants that grew in the neutralized soil. The plants that grew in the Dunkirk soil contained more phosphorus than those that grew in the Volusia soil. The amounts absorbed under acid and neutral conditions were nearly equal in the Dunkirk soil. Considerably more potassium was taken up by the plants receiving mineral nutrients and manganese than by either the untreated or the soils to which only calcium carbonate was added.

In the foregoing experiments with soils, the purpose was to determine the effect produced on the growth of plants in an acid and a neutral soil to which was added graduated amounts of manganese sulphate. It is readily apparent, therefore, that while such methods of experimentation are very important from an economic standpoint, they do not afford the desired proof to determine whether or not manganese is essential in the plant's economy.

The preparation of a medium that will, when the test is applied, show no manganese, requires certain procedures and precautions which, in so far as the writer has been able to discover, have not previously been taken into consideration in experiments planned to show the effect the absence of this element may have on the growth of plants. Tests for manganese in many different samples of chemical reagents which are used for plant nutrients show that manganese-free chemicals are rarely if ever found. So intimate is the association of manganese with iron that a manganese-free iron compound could not be purchased from dealers in chemicals and it was necessary to prepare it in the laboratory. Therefore in order to eliminate all possible sources of contamination with manganese it is necessary to test and purify all the reagents that enter the nutrient medium. Even though manganese has been eliminated from the nutrient medium it is necessary to grow plants until they approach maturity before any definite conclusions can be reached with respect to the effect of this element on their growth. The seeds of those plants with which the writer has carried on experiments apparently contained enough manganese to maintain a normal growth for the first six or eight weeks.

After having procured reagents that were proved by chemical tests to be free from manganese, a Knop's nutrient solution was made for water culture experiments.

Alaska garden pea seeds were germinated on moist cheesecloth in a porcelain-lined pan. When the seedlings were of the proper size they were transferred and held in place by means of plugs of cotton in holes made in squares cut from boards which were made to fit over the mouths of well-glazed one-half gallon earthenware jars. An attempt was made to waterproof the boards by keeping them submerged in paraffin kept near the boiling point for several hours. Although the squares of wood afford excellent means of support for the plants, they proved to be unsatisfactory in these and in previous experiments from the fact that the lower surface of the board, which is close to the top of the nutrient solution, affords favorable conditions for the growth of molds which, unless daily attention is given to washing them away, very soon attack the roots of the plants.

More recently supports have been made of well-glazed earthenware and no trouble from molds has been experienced.

The earthenware jars were filled nearly to the mouth with the Knop's solution and when the covers containing the seedlings were on, the roots were submerged in the solution. The pots containing the plants were kept at a suitable temperature for the growth of the peas in the greenhouse and the nutrient solutions were changed twice each week. The water lost by transpiration in the meantime was replaced with distilled water, thus keeping the concentration approximately constant.

Previous to starting the experiment new pots were obtained and tested for soluble manganese in the following manner: Three of the new pots were taken at random and filled to near the mouth with a mixture of equal parts of 1:1 nitric and hydrochloric acids and placed over holes on a water bath and kept at the temperature of boiling water for 48 hours. The mouths of the pots were covered with watchglasses during the digestion. The solutions were then transferred to porcelain dishes and brought to dryness. The residues were tested for the presence of manganese, and only a slight trace was found from any of the pots. The writer considers this a negligible source of manganese as it was thought

that even the trace found in the acid extraction would not be available to the plants growing in a Knop's solution.

Twelve Alaska pea seedlings were placed in each of four jars containing a Knop's solution. At the beginning of the experiment 1 part of manganese in the form of the sulphate to 1,000,000 parts of the solution was added to each of two of the pots. After the plants had been growing three or four weeks the concentration of manganese was increased to five parts per million of the Knop's solution. One cubic centimeter of a manganese-free ferric chlorid solution which contained 0.001 gm. iron was added to each of the pots at the time the solutions were changed.

During the first four or six weeks of growth there was little or no difference in the size or appearance of the plants. Therefore the plants which received no manganese except that contained in the seed began to show effects which can not be accounted for in any other way except for a lack of this element. The first effect to be observed was that the young buds as they unfolded were yellowish instead of a normal green color and later became flecked with small specks of brown, which was not observed at any time on the plants receiving manganese. The etiolated condition became more pronounced as the time progressed and finally resulted in the top branches dying back. The green and dry weights of the 12 plants that grew in each of the 4 pots were determined.

The average dry weights of the pea plants show an increase of 49.47 per cent in the total dry matter produced in the plants which were grown in the presence of manganese when compared with those plants that grew in a Knop's solution to which no manganese was added. There was also an increase of 24.63 per cent in the mineral nutrients assimilated, exclusive of nitrogen, in the plants that grew in the Knop's solution containing manganese. The plants which did not get any manganese contained a little more nitrogen than those to which manganese was added. The plants from which manganese was withheld contained only traces of this element, which was no more than could have been derived from the seeds from which the plants grew. It therefore appears that the trace of manganese derived from the seeds of the plants was not sufficient to maintain a normal metabolic process during the latter part of the time the plants were growing.

The duplicate sets of plants were combined to make a composite sample for chemical analysis and the results are given in Table V.

TABLE V.—Analyses of pea, soybean, corn, and cowpea plants grown in media free from, and containing manganese (results expressed of the moisture-free material)

	Ash (crude).	Iron (Fe).	Man- gane- se (Mn).	Cal- cium (Ca).	Mag- nesium (Mg).	Potas- sium (K).	Phos- phorus (P).	Nitro- gen (N).
Alaska pea plants:	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>
Manganese.....	12.85	0.041	0.179	1.85	0.50	2.05	0.58	2.97
No manganese.....	15.41	.040	Trace.	2.41	.98	2.71	.68	4.71
Soybean leaves:								
Manganese.....	15.00	.051	.032	.80	.56	1.58	.62	3.42
No manganese.....	15.99	.094	Trace.	.82	.62	1.11	.48	3.85
Soybean stalks:								
Manganese.....	13.07	.036	.010	.57	.37	1.08	.45	1.69
No manganese.....	16.67	.033	None.	.68	.39	1.35	.46	2.21
Cowpea plants:								
Manganese.....	17.24	.046	.036	1.70	.54	5.56	.72	2.57
No manganese.....	20.14	.065	.003	1.32	.61	7.88	.51	4.51
Corn leaves:								
Manganese.....	8.71	.029	.040	.23	.31	3.80	.40	.22
No manganese.....	10.28	.034	.001	.24	.36	4.07	.35	1.47
Corn stalks:								
Manganese.....	6.37	.014	.003	.08	.13	3.02	.42	.77
No manganese.....	8.24	.011	Trace.	.10	.13	3.52	.37	.95

## SAND CULTURE EXPERIMENTS

A quantity of quartz sand of medium sized grains was obtained for sand cultures. The sand, from external appearance, was good quality glass sand. Upon digesting 1,000 gm. in a mixture of 1:1 hydrochloric and nitric acids on a hot water bath for several hours, filtering out and washing the residue free of acids, it was found upon testing portions of the filtrate that the sand had contained small amounts of iron, manganese, zinc, arsenic, calcium, and magnesium. The residue of sand was snow white, except for a few black particles, which resisted further treatments of strong acids to dissolve them. A small amount of the black particles were separated, ground in an agate mortar, and fused with potassium bisulphate. The fused mass was extracted with dilute sulphuric acid and tested for manganese, but none was found.

Several hundred pounds of the sand were then washed with a mixture of nitric and hydrochloric acids and afterwards with distilled water until free of chlorids, as shown by a test with silver nitrate. The sand was then transferred to large, shallow, porcelain-lined agateware pans and dried. After the sand was dry 4,000 gm. portions were weighed into clean 1-gallon earthenware pots.

The sand prepared in this way possessed rather strong absorptive powers when wet with a Knop's nutrient solution. It, therefore, was necessary to saturate the absorptive capacity of the sand with respect to the plant nutrients before plants could be grown. This was accomplished by adding the following amounts of manganese-free mineral nutrients to each 1 gallon of dry sand (4,000 gm.); 25 gm. of  $\text{CaCO}_3$ , 10 gm. of  $\text{K}_2\text{HPO}_4$ , 10 gm. of  $\text{Ca}(\text{NO}_3)_2$ , 5 gm. of  $\text{MgSO}_4$ , 3 gm. of  $\text{KCl}$  and 3 gm. of  $\text{Fe}$  in the form of a suspension of  $\text{Fe}(\text{OH})_3$ . These chemicals were all free from manganese and pots of sand receiving this treatment served as the controls. Equal numbers of other pots of the sand received 2 gm. each of manganese in the form of  $\text{MnCO}_3$  and the above-mentioned nutrients. The mineral nutrients and the sand were thoroughly mixed by hand in a large porcelain-lined pan and returned to the respective pots. Equal numbers of plants of soybeans, cowpeas, and sweetcorn were grown in the purified sand, with and without manganese, until they approached maturity. They were then harvested and the dry weights determined and chemical analyses made of the plant material. The results are given in Tables V and VI.

TABLE VI.—*Dry weights of the plants*

Species of plant.	Manganese added.	No manganese added.	Increase due to manganese.
	<i>Gm.</i>	<i>Gm.</i>	<i>Per cent.</i>
Alaska peas (grown in water cultures).....	28. 10	18. 80	49. 5
Soybeans.....	10. 80	12. 25	61. 6
Cowpeas.....	26. 50	11. 30	134. 5
Corn.....	72. 00	59. 00	22. 0

All the plants which grew in the pot cultures containing manganese made a very much better growth than did those from which manganese was withheld. The most striking result was obtained with cowpeas, in

which the increase in dry weight of the plants receiving manganese was 134.5 per cent. All the leguminous plants from which manganese was withheld made a normal growth for the first six or eight weeks; thereafter the young buds and leaves as they unfolded were etiolated and brown specks developed on the etiolated leaves later. After this condition had developed but very little growth was made, and the young and tender parts of the plants died back, while the plants to which manganese was added made a normal growth and no chlorosis developed during their growth. With corn, the only apparent effect produced on the growth of the plants was the failure in the production of dry organic matter. No chlorosis developed on the leaves of the corn, which was harvested after the plants had produced tassels. From these results it appears that leguminous plants are more sensitive towards the lack of manganese than are the nonlegumes.

In the cowpea and corn plants to which no manganese was added, enough of this element was present for a determination. In the other plants to which no manganese was added only a trace of manganese could be detected.

The stalk of the soybean plants which grew in the absence of manganese was the only material in which no manganese could be detected. The plants which grew in the pot cultures to which manganese was added contained a normal amount of this element.

More recent experiments with manganese-free sand cultures at the Kentucky Agricultural Experiment Station have shown the necessity of manganese in both nonleguminous and leguminous plants.

Plate 2, A, shows in a very striking way the beneficial influence of manganese on oats. The plants on the left had no manganese added to the sand in which they grew, while those on the right grew in the presence of manganese.

Plate 2, B, shows Canada field peas. The plants on the left received no manganese in the sand in which they grew, while to those on the right manganese was added, all other conditions being the same.

Sand cultures were carried out with the following species of plants: Wheat, oats, peas, cowpeas, beans, lettuce, tomatoes, onions, spinach, cabbage, carrots, radish, and clover, and similar effects were observed as those described and shown in Plate 2, A, B.

#### SUMMARY

(1) In the experiments with acid soil which contained approximately one-tenth of 1 per cent of manganese it was found that only small amounts of the total manganese were soluble in water. Applications of more manganese, in the form of the sulphate, to the acid soil caused a decrease in the yields of the crops, whereas like quantities of this compound, when applied to different portions of the same soil, after addition of calcium carbonate, caused an increase in the yields of other plants of the same species.

(2) The occurrence of soluble salts of manganese in an acid soil may be one of the causes of toxicity in such soils as exhibit toxic effects. An excess of manganese sulphate in a soil renders it sterile with respect to the growth of plants. Calcium carbonate in the soil causes a diminution in the toxic effects produced by an excess of manganese sulphate.

(3) In order to demonstrate whether or not manganese is essential for the normal growth of plants, it is necessary that great care should

be taken in the preparation of a manganese-free medium in which to grow plants.

(4) ✓ Manganese is intimately associated with compounds of iron, phosphorus, and calcium, and since very small amounts of this element are required for the growth of plants it is quite probable that the contamination of plant nutrients with manganese has hitherto been an unrecognized source of error in determining the necessity of this element in plant economy.

(5) ✓ Apparently leguminous plants are more sensitive to the lack of manganese than are the nonlegumes; however, further data obtained by growing nonleguminous plants for more than one generation in a manganese-free medium may be necessary to prove this point.

(6) ✓ A very small amount of manganese is required for the normal growth of plants.

(7) ✓ Seeds of the plants tested (radish, soybean, cowpea, field pea, and corn) do not contain enough manganese for the growth of the plant to maturity.

(8) ✓ The seeds of some plants contain enough manganese to maintain a normal development for the first four or six weeks of their growth; therefore experiments conducted for a shorter time in a manganese-free medium are not likely to give any indications as to the necessity of this element in the growth of plants.

(9) ✓ The lack of manganese affects the production of the dry matter in plants, thus indicating that it has some very important function in carbon assimilation.

(10) ✓ The etiolated condition of the young and tender leaves and buds obtained in well-controlled experiments indicates that manganese has a function in the photosynthetic process and the formation of chlorophyll.

(11) ✓ Manganese apparently is essential for the normal growth and development of plants.

(12) Further investigations on this subject are under way in which an attempt will be made to show the relation of manganese to other important phases of plant and animal life.

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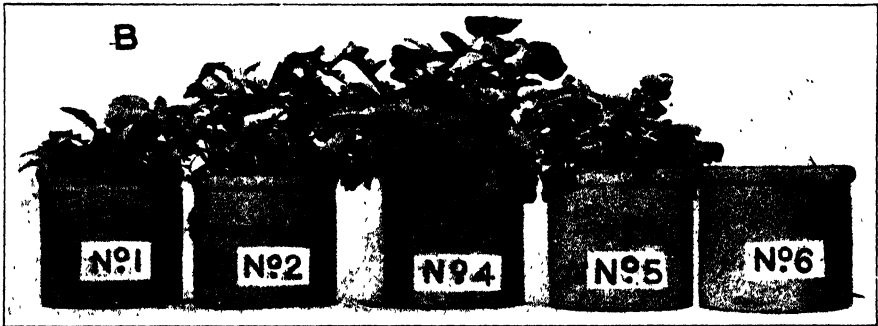


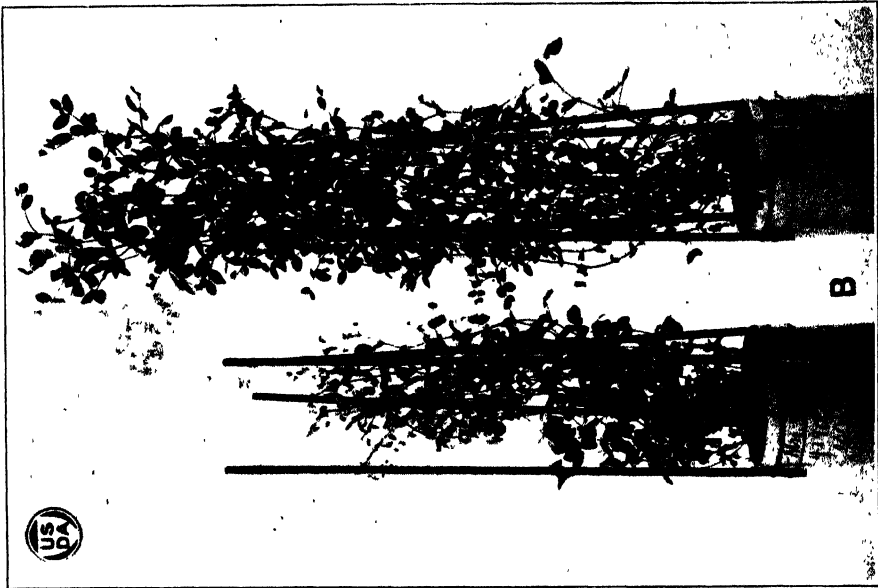
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**PLATE I**

- A.—Radish plants in Volusia silt loam;  $\text{MnSO}_4$  and plant nutrient added to No. 3, 4, and 5.  
B.—Volusia silt loam with  $\text{CaCO}_3$ ;  $\text{MnSO}_4$  and plant nutrients added to No. 4, 5, and 6.





**PLATE 2**

**A.—Oats in sand; left, no Mn added; right, Mn added.**

**B.—Canada field peas in sand; left, no Mn; right, Mn added.**



# SWEET CLOVER INVESTIGATIONS<sup>1</sup>

By RAY E. NEIDIG, *Chemist*, and ROBERT S. SNYDER, *Associate Chemist, Idaho Agricultural Experiment Station, University of Idaho*

## COMPOSITION OF SWEET CLOVER HAY AND SILAGE

Sweet clover has become an important crop in Idaho, not only from the standpoint of feeding value, but also because it is finding a place in a proper rotation system of cropping which is so necessary for diversified farming. Sweet clover is a legume that is comparatively easy to grow in all sections of the Northwest. Its value as a soil improver is rapidly becoming known to progressive farmers both in the humid and semiarid regions. As a pasture crop, sweet clover is finding a much needed place on the farms of the Northwest. Its resistance to drought and its great productiveness under the most adverse conditions warrants a place on every farm. Recent indications are that sweet clover is an excellent crop for alkali districts when the alkali concentration is too heavy for the ordinary hay crops.

Since sweet clover is finding a place in a permanent agricultural system, the following investigation was planned to determine the feeding value at various stages of growth, and also to determine the possibility of utilizing the crop for silage whenever conditions did not warrant making hay. It appeared to the writers that owing to the heavy yields of sweet clover that can be obtained on lands that are not adaptable to the growing of corn or sunflowers, it would prove an excellent substitute for these crops for silage if it could be satisfactorily siloed in Idaho. Consequently the two varieties (*Melilotus alba*) or white, and (*Melilotus officinalis*), or yellow sweet clover, were studied. These two varieties were grown by the Agronomy Department in 1920, and were available for use in these investigations.

### STAGES SELECTED

In studying the varieties, two stages were selected for the yellow sweet clover. The first stage was the time when the plant was in blossom. At this stage the plant was 37 inches in height. In all samplings the yield of a definite area was determined, and from this the acre yield was calculated. When the plant was partly in blossom and partly in seed it was cut, and the authors called this the second stage.

The samplings of the white sweet clover were made in three stages: First, before the plant was in blossom and when it was 49 inches in height; second, when the plant was beginning to blossom; and, third, in full blossom and when a few seeds were formed.

The samples were prepared for analysis and for silage by cutting in small pieces in a small silage cutter. A portion of the cut sample was air dried and then analyzed for protein, ether extract, fiber, ash, and nitrogen-free extract. The remaining portion of the sample was used for silage.

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<sup>1</sup> Accepted for publication Nov. 6, 1922.



## PREPARATION OF THE SILAGE

Quart milk bottles were used for making the sweet clover silage, since these had been used on other types of silage experiments and found to be very well suited for such work.

The technic of silage making was as follows: A quart milk bottle was packed with the freshly-cut sweet clover and, if lacking in moisture, sufficient water was added to raise the moisture content to 75 or 80 per cent. A large rubber stopper containing a glass tube was placed in the bottle very tightly. This tube was bent at an angle so that its outer end was inserted into a beaker containing mercury. This arrangement allowed the fermentation gases, chiefly carbon dioxid, to escape from the bottle, but did not allow access of air to the silage. At the end of a few weeks, when the major fermentation had been completed, the bottles were opened and the silage examined. The acidity of the silage was determined, also the protein, ether extract, fiber, ash, and nitrogen-free extract.

## METHODS USED

In determining the composition of the sweet clovers at various stages of growth, the methods described by the Association of Official Agricultural Chemists were used.<sup>2</sup> The results on the composition are given for the various stages in Table I.

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<sup>2</sup> ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. Revised to Nov. 1, 1919. xii, 417 p., 18 fig. Washington, D. C. 1920. Bibliographies at end of chapters.

TABLE I.—Composition of sweet clover and sweet clover silage

Variety.	Stage of cutting hay.	Date.	Height.	Acre yield.	Moisture.	Wet basis.					Anhydrous basis.				
						Crude pro-tein.	Ether extract.	Crude fiber.	Ash.	Nitro-gen-free extract.	Crude pro-tein.	Ether extract.	Crude fiber.	Ash.	Nitro-gen-free extract.
Yellow.	In blossom.	July 2, 1920	In.	Tons.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
	In blossom and partly in seed.	July 13, 1920	37	11.7	73.5	5.56	0.85	6.8	2.99	10.3	21.09	3.21	25.66	11.28	38.86
	(Silage made from the above two stages of yellow sweet clover):		37	10.9	69.5	5.00	.63	9.99	2.90	11.92	16.59	2.67	32.78	9.49	39.07
White. Original Hay.	First stage.				75.6	4.50	.63	7.48	2.60	9.19	18.44	2.59	30.65	10.61	27.71
	Second stage.				72.4	4.38	.49	9.31	2.78	10.64	15.87	1.78	33.71	10.06	38.58
	Before blossoming.	July 2, 1920	49	21	75.4	5.38	.45	7.59	2.65	8.53	21.87	1.83	30.83	10.75	34.72
	Beginning to blossom.	July 14, 1920	60	14	73.4	5.06	.55	9.01	2.16	9.82	19.02	2.06	33.83	8.12	37.97
	In full blossom.	July 22, 1920	60	16.8	69.4	4.25	.57	11.64	2.34	11.80	13.99	1.87	38.02	7.64	38.48
	(Silage made from the above stages of white sweet clover):														
	First stage.				77.16	3.75	.51	7.83	2.09	8.66	16.42	2.23	34.20	9.16	37.99
	Second stage.				75.32	3.69	.23	8.52	1.99	10.25	14.95	.93	34.50	8.05	42.57
	Third stage.				70.64	4.00	.47	10.75	2.10	11.92	15.07	1.61	30.57	7.30	38.79

## ACIDITY OF SWEET CLOVER SILAGE

## METHODS OF OBTAINING SAMPLES AND DETERMINING ACIDITY

The silage was removed from the containers and 50 gm. were immediately weighed out and dried at 100° C. to determine the moisture content. The remaining silage was placed in a hydraulic press and the juice pressed out. Then 100 gm. of the juice was placed in a vacuum steam distilling flask, together with 5 cm. of normal sulphuric acid. The volatile acids were separated from the nonvolatile acids by distilling in a current of steam in a partial vacuum. Four liters of the distillate were collected, which contained all the volatile acids. The distillate was neutralized with one-tenth normal barium hydroxid and evaporated to a small volume. The volatile acids were then freed from the barium salts by the addition of the theoretical amount of sulphuric acid. After filtering the barium sulphate, the solution was made up to volume and the volatile acids were quantitatively determined, using the methods described by one of the authors in a former publication.

Total acidity was determined by titrating 10 gm. of expressed juice with one-tenth normal barium hydroxide. Nonvolatile acid was determined by subtracting the centimeters of volatile acids found in 100 gm. of silage juice from the total acidity determination. The difference was calculated as lactic, or nonvolatile acid. The results of the volatile and nonvolatile acids are given in Table II, on the silage both with and without moisture.

TABLE II.—Acidity of sweet clover silage.

Variety.	Stage of cutting.	Height of sweet clover.	Acre yield.	Amount of acids in 100 gm. wet silage.			
				Acetic.	Pro-pionic.	Lactic.	Total.
		<i>In.</i>	<i>Tons.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>
Yellow ..	In blossom .....	37	11.7	0.371	0.011	1.399	1.781
Do ..	In blossom and partly in seed .....	37	10.9	.094	.054	.853	1.601
White .....	Before blossoming .....	49	21	.515	.013	1.353	1.881
Do .....	Beginning to blossom .....	60	14	.333	.021	1.250	1.604
Do .....	In full blossom .....	60	16.8	.332	.023	1.642	1.997

Variety.	Stage of cutting .	Height of sweet clover.	Acre yield.	Amount of acids in 100 gm. dry silage.			
				Acetic.	Pro-pionic.	Lactic.	Total.
		<i>In.</i>	<i>Tons.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>
Yellow .....	In blossom .....	37	11.7	1.520	0.043	5.728	7.291
Do .....	In blossom and partly in seed .....	37	10.9	2.516	.020	3.091	5.627
White .....	Before blossoming .....	49	21	2.167	.06	5.699	7.926
Do .....	Beginning to blossom .....	60	14	1.348	.09	4.063	5.501
Do .....	In full blossom .....	60	16.8	1.133	.08	5.599	6.812

## DISCUSSION

A comparison between the white and yellow sweet clover shows that the former is the higher yielding variety. It must be borne in mind that both varieties were grown side by side, hence were subjected to the same climatic and soil conditions. The white sweet clover produced a taller and bushier plant than the yellow, which accounts for its greater yield.

In both varieties the first stage contained the larger percentage of water, as was expected. The later cuttings were higher in dry matter. The only significant fact shown (Table I) is that the nitrogen or crude protein decreased and the crude fiber increased, the longer the sweet clover was allowed to grow. This is true for both varieties and clearly demonstrates that if the sweet clover is to be used for hay the early cutting should be chosen rather than the later stage. For silage purposes this conclusion is equally true, for there is slightly less crude fiber and a slightly higher percentage of protein in the earlier stages of cutting.

The acidity determinations show that a normal silage fermentation takes place when sweet clover is siloed. The silage samples all had the characteristic silage acids and odor and were classed as excellent silage.

It is not the purpose of this paper to advise the growing of sweet clover for silage rather than corn or sunflowers, but under certain conditions it is thought that sweet clover can be so used to advantage. In its use as a pasture crop one of the chief difficulties encountered is to pasture sufficient stock upon the sweet clover to keep down the growth. In many cases the sweet clover grows so rapidly that the growth exceeds the pasture requirements and a portion of the sweet clover attains a considerable height. It is thought that in such conditions, where the crop may not make the best hay, it can well be used for silage, since the stalks, if they have been allowed to become too mature, will soften up materially in the silo. Two purposes have been accomplished when a sweet clover crop from pasture land has been used in this manner. First, the food value of the excess growth has been saved and, second, the pasture has been improved by removing the excess growth, and new tender shoots will appear.

The fact that sweet clover is a legume and acts as a soil improver and gives a comparatively high yield of green material under the most adverse conditions of drought, tends to make it a promising crop for the silo in many parts of the Northwest.



# GROWTH AND COMPOSITION OF ORANGE TREES IN SAND AND SOIL CULTURES<sup>1</sup>

By H. S. REED, *Professor of Plant Physiology*, and A. R. C. HAAS, *Assistant Professor of Plant Physiology, College of Agriculture, University of California*

The limited control over the factors of nutrition operating in the field (10)<sup>2</sup> (4) has, perhaps, contributed in a large measure to the heterogeneous array of causes assigned to specific effects. Before any explanation of so-called nutritional disturbances of citrus trees can be accepted, they must be produced experimentally under conditions admitting of scientific analysis. The method subsequently described for growing citrus trees under controlled conditions should make such an analysis possible. The present paper deals with a comparison of the growth and composition of trees when grown in sand and in soil cultures.

It is important to determine, first, whether citrus trees can be grown successfully for several years in sand cultures receiving only inorganic salt solutions; second, whether the growth obtained in sand receiving a nutrient solution (which has been shown to be well suited to the growth of barley) compares favorably with that obtained in soil receiving occasional additions of the same nutrients; and, third, how the composition of the trees grown in sand compares with that of the trees grown in soil.

The importance of the method and the successful results thus far obtained seem to justify a brief description of the way in which these cultures were installed and cared for. The procedure to be described was adopted only after considerable study and experimentation.

As experiments upon trees are usually of long duration, adequate protection and cultural attention are quite essential. A screened enclosure with suitable windbreaks provided the necessary protection against animals and destructive winds. The experiments were conducted in sheet-iron tanks and in large galvanized iron cans. The former were sunk in the ground; the latter were placed in trenches for protection from the direct rays of the sun.

The trenches were 28 inches to 30 inches wide, about 3 feet deep, and were lined on both sides with boards. In the bottom of each trench were two 2-inch by 4-inch pieces of redwood extending the entire length of the trench, which served as supports for the cans and prevented rusting. Thus far it has not been found necessary to provide wooden covers for the trenches. The necessary protection against rain has been obtained by placing frames of roofing paper on wooden trapezes swung by wires from the roof of the enclosure.

Each of the galvanized iron cans (Pl. 1, A) is about 20 inches in diameter and about 26 inches in depth. In order to have a means of weighing the cans, three wrought-iron lugs  $\frac{1}{4}$  inch by 1 inch, each bearing a three-eighths inch hole at the top, were fastened by bolts to the inside of the heavy upper rim. The lugs were equally spaced around the rim and protruded through holes cut in the lid (Pl. 1, B).

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<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 813-814.

A 4-inch hole was cut in the center of each lid to accommodate the tree. About midway between this center hole and the lid margin, four equally spaced  $1\frac{1}{4}$ -inch holes were cut, through which solutions could be poured. These holes were corked when not in use. The inside surface of the can was given a heavy coat of asphalt paint.

The sand used in the cans upon mechanical analysis showed the following percentage composition:

Size by screen (meshes per inch):	Per cent
Larger than 20 mesh.....	0.3
20 to 40.....	7.8
40 to 60.....	54.1
60 to 80.....	21.6
80 to 100.....	16.0
Smaller than 100.....	

Chemical analysis of the sand showed 99 per cent silica, 0.2 per cent volatile matter, and 0.8 per cent total water-soluble solids (other than silica or volatile matter), much of which was iron.

A 1-inch pipe, inserted through the lid and extending to the base of the can, provided a means for removing drainage water. About it and to a depth of 4 to 6 inches was placed a well-compacted layer of pure quartz rock crushed to about one-fourth to one-half inch in diameter, to prevent the sand from blocking the drainage system. The sand was added gradually and thoroughly moistened and settled before further additions, care being taken to avoid the use of excessive amounts of water. When the can was filled with sand to within about 2 inches from the top, the excess of water used in filling the can was at once removed through the outlet pipe.

The drainage water was removed by means of powerful air-suction (7) (Gardner-Rix vacuum pump run by one-half horsepower motor, with a capacity of 9 cubic feet per minute), six to eight cans being usually drained in one operation.

A slender iron tube (Pl. 1, B) was inserted through the drainage pipe to the bottom of the can whenever it was necessary to remove drainage water. As a means of excluding sand the lower end of the drainage tube was covered with a piece of fine tin screen. Excellent results over a period of years are readily obtained if the drainage water is removed as rapidly as it enters the drainage pipe. Plate 1, B, shows one of the drainage bottles removed from its wooden rack and raised so as to show the general scheme used in removing the drainage water.

The air outlet from each drainage bottle is provided with a gas cock by means of which the rate of flow into each bottle can be regulated.

A 2-year-old Valencia orange tree was planted in each can after the soil was thoroughly removed from the roots by repeated washing. The root system was pruned until only a small part remained. The upper portion of the tree was also pruned (Pl. 1, A); the lateral shoots and the leaves were removed and the cut surfaces covered with asphalt paint. Where it was not desired to exclude calcium from the culture, the trees were given a coat of whitewash. Where whitewash was not applied, a cylinder of wire netting was placed about the tree and the east and south sides were protected with cheesecloth attached to the wire. When the trunk was shaded by new shoots, the cloth protector was removed, preferably during cloudy or foggy weather. After the tree was planted in the sand and the lid was placed on the can a piece of white oilcloth, with a hole in the center, was slipped down over the tree trunk and was fastened to the lid with asphalt paint. A thin wad of cotton was placed

between the oilcloth and the tree. A cylinder of heavy brown wrapping paper was placed about the base of each trunk to give added protection from sunburn.

Where soil was employed the cans were prepared as stated before, which made possible the removal of salts while the tree was in the soil. Wilting-point determinations were made on the soils and they were then brought to a suitable water content prior to being gently compacted in the cans.

In the case of sand, additions of carbon-treated distilled water were continued until the first indications of drainage water appeared in the drainage pipe. Air suction was then applied. Nutrient solutions (6 to 9 liters or more) made up with carbon-treated distilled water were then added and the drainage continued until complete. If drainage water appeared later in the bottom of the cans, the air-suction was again applied until all the free solution had been removed. The problem of drainage was subsequently simplified by providing a means for gravity drainage. The cans were placed on supports over the trenches and a short iron pipe was inserted into the bottom of each can. As drainage water percolated to the bottom of the can, it dripped through the short pipe into a bottle standing in the trench. This arrangement is decidedly advantageous because it precludes the accumulation of water in the bottom of the can and also materially lessens the amount of labor required in caring for the cultures. The cans were shielded from the direct rays of the sun by housing them in a wooden box. The treatments were applied every one to three weeks as conditions required. During hot weather a small tree growing in sand (about 225 pounds of moist sand) may require from 24 to 30 liters of distilled water each 20 days. A Barnstead still capable of giving 5 gallons of distilled water per hour, with a block-tin-coated tank capable of storing 2,000–2,500 liters of distilled water, and 24 large carboys for treating the distilled water with carbon black together with filtering facilities, have made it possible to maintain in operation 136 trees in cans as well as several trees in tanks of sand.

In the case of cans of soil when drainage water may not be desired, the weighing device patterned after that of Briggs and Shantz (2) is used (Pl. 1, C). A windlass with wire rope and pulleys is mounted on a frame work of 2-inch galvanized-iron piping to which wide swivel castors are attached. Two spring scales with circular disks, 500 and 600 pounds capacity, respectively, are used for bringing the cans to constant weight. As the trees develop in cans of soil, it is necessary to make allowance for the increased weight of the tree in order to maintain suitable moisture content of the soil. The criteria used for this purpose are, appearance of the tree, presence or absence of drainage water, and appearance of the surface of the soil upon slightly raising the lid, or preferably by examining a sample of the soil. It is a good practice occasionally to raise the lids of the cans of soil to ascertain whether channels have been formed in the surface layers. It is best to weigh the cans frequently, since if large additions of solution are required, channels may appear throughout the soil and drainage water may appear without much of the soil being appreciably above the wilting point.

In order to carry on experiments with trees for a longer period of years, containers of a larger capacity than that of the cans are necessary. Tanks of two sizes are being used. The smaller are 3 feet 8 inches in diameter by 4 feet deep (Pl. 2, A), the larger are 7 feet 8 inches in diam-



eter by 4 feet 4 inches deep (Pl. 3, A). The bottom of each tank tapers toward the center, which is 6 inches deeper than the circumference.

The center of the bottom of each tank bears a perforated brass plate, beneath which is attached a 4-inch elbow that connects with 2-inch galvanized-iron piping leading to a trench. Each tank has its individual drainage outlet. Crushed quartz rock was placed in the bottom of each tank above which is placed sand or soil, as the case may be.

The nutrient solution employed in the present experiments was that used by Hoagland (5) in the culture of barley. Filtered carbon-treated distilled water was used whenever water was required. The stock solutions were made up as follows:

Solution A.	Gm.
KNO <sub>3</sub> .....	1,200
MgSO <sub>4</sub> +7H <sub>2</sub> O.....	1,800
NaCl.....	55
H <sub>2</sub> O to give volume of 18 liters.	
Solution B.	
Ca(NO <sub>3</sub> ) <sub>2</sub> +4H <sub>2</sub> O....	2,600
H <sub>2</sub> O to give volume of 18 liters.	
Solution C.	
KH <sub>2</sub> PO <sub>4</sub> .....	900
H <sub>2</sub> O to give volume of 18 liters.	
Solution G.	
MnSO <sub>4</sub> +4 H <sub>2</sub> O.....	0.406
H <sub>2</sub> O to give volume of 2 liters.	

The nutrient solution was prepared by using the following amounts of the stock solutions: 55 cc. of solution A, 65 cc. of solution B, 30 cc. of solution C, and 20 cc. of solution G, made up to 10 liters with distilled water.

The nutrient solution as thus made had a  $P_H$  of 5.2 and an osmotic pressure of 0.728 atmospheres. The composition of the nutrient solution was as follows:

*Parts per million.*

NO <sub>3</sub>	SO <sub>4</sub>	K	Ca	PO <sub>4</sub>	Mg	Cl	Na	Fe	Mn	Total
718	216	185	159	105	54	10	7	1	0.1	1455

The iron was not added to the nutrient solutions until they were ready to be added to the cultures. As a rule 5 to 10 parts per million of Fe were used in the nutrient solution, the plan being to have sufficient Fe present, as indicated largely by the appearance of the tree. Small quantities of ferric tartrate were subsequently added whenever the cultures were irrigated.

The trees were planted on May 21, 1920, and were removed September 20, 1921. Trees 1 and 2 were typical of the sand cultures (1-5), and tree 85 was typical of the soil cultures (84-88). The tree in can 2 was much smaller than the others in its series at the start, although it made excellent growth.

Plate 2 B, is a photograph of trees 1, 2 and 3 taken August 20, 1921. The photograph indicates that citrus trees can be grown in pure sand to which no organic matter has been added, except the small amount as iron tartrate. Plate 4 shows the leaves of tree No. 3 which were typical of the series, being dark green in color and giving no indications of malnutrition.

This is of interest in connection with the results (1) of field studies in which it was found that low humus content of the soil was associated with the mottling of orange trees and also that applications of organic

fertilizers tended to reduce the amount of mottling. Although the authors have grown 23 orange trees in sand cultures and 5 in soil receiving various concentrations of an inorganic nutrient solution, no typical mottle-leaf has been found as yet on any of the trees.

A word of caution is here necessary as regards the choice of orange trees for sand cultures. It seems best to use trees which are free from mottle-leaf, although trees which previously bore mottled leaves may present no difficulties after their behavior is once known. When mottled trees have been used in sand cultures receiving Hoaglands solution, and the leaves, shoots, rootlets, etc., removed as before stated, the first cycle of growth may bear a few mottled leaves most of which may recover, but the subsequent cycles have thus far been free from any evidence of mottling.

In order to observe the effect of the sand on the  $P_H$  of the solution, some of the nutrient solution was placed in liter Erlenmeyer flasks closed with rubber stoppers. Sand was added to one flask, shaken, and allowed to stand from December 29, 1920, to January 24, 1921. The  $P_H$  of the nutrient without sand had not changed, while that in contact with sand had increased from 5.2 to 5.9. Other culture solutions treated in this manner gave similar results. Shive (9) has reported that the reaction of his nutrient solution was not markedly altered by contact with unwashed sand. He finds that sand has an initial adsorptive effect but this is soon satisfied in sand cultures where the nutrient solution is renewed frequently. The increase in the  $P_H$  of the solution in contact with the sand was probably due to chemical reactions and not to adsorption.

The adsorptive property of unwashed sand probably was due to the very finely divided colloidal or semicolloidal material which was removed from the sand in the process of washing. The  $P_H$  values of percolates from the cans were determined from time to time to learn whether material changes ensued after prolonged contact with the sand and tree roots. The results of several determinations on percolates from cans 1 and 2 are given in Table I. The first two determinations showed the increase when a nutrient solution having an initial  $P_H$  of 5.2 was allowed to percolate through the sand of one of the cans. The other determinations show the changes in  $P_H$  when distilled water was allowed to percolate through the sand. These determinations are more variable, owing in part to the fact that the distilled water washed out varying quantities of the residual nutrient solution. In every case the  $P_H$  of the percolate tends to move in the direction of neutrality.

TABLE I.— $P_H$  values of nutrient solution and percolate from trees grown in sand cultures

Date.	Application.	Amount of percolate collected.	Tree No.	$P_H$ of percolate.
1920:		Cc.		
Nov. 2.....	Nutrient solution.....	100	1	6.8
Do.....	do.....	100	2	6.7
Nov. 29.....	Distilled water.....	500	1	7.25
Do.....	do.....	500	2	6.6
1921:				
Mar. 28.....	do.....	500	1	5.4
Do.....	do.....	500	2	6.5
Apr. 20.....	do.....	10	1	5.9
Do.....	do.....	10	2	6.7

The osmotic pressure of the sap of leaves from trees in the sand cultures was 20.80 atmospheres and that of leaves from the soil cultures was 21.03 atmospheres. The  $P_H$  of the former was 6.00 and that of the latter 5.80. We can not regard these differences as very significant.

Can 85, as well as the other cans of the series 84-88, inclusive, contained soil obtained from an uncultivated hillside near Riverside, Calif.

The hygroscopic moisture content of the soil was determined and the soil was brought to a suitable moisture content.

The cans of soil were brought to a given weight at frequent intervals by the addition of distilled water or nutrient. During the experimental period (May 21, 1920, to September 20, 1921) can 85 received the following additions of salts.

Salts.	Amounts added.
	Gm.
KNO <sub>3</sub> .....	9. 89
MgSO <sub>4</sub> +7H <sub>2</sub> O.....	14. 85
NaCl.....	0. 46
Ca(NO <sub>3</sub> ) <sub>2</sub> +4H <sub>2</sub> O.....	25. 27
KH <sub>2</sub> PO <sub>4</sub> .....	4. 05
Fe <sub>2</sub> (C <sub>4</sub> H <sub>4</sub> O <sub>6</sub> ) <sub>3</sub> .....	0. 17
MnSO <sub>4</sub> +4H <sub>2</sub> O.....	0. 0108

Plate 3, B, shows a typical tree in the soil series (84-88). The growth in sand compared very favorably with that in soil.

In November, 1920, after the addition of distilled water to cans 1 and 2, the first 500 cc. of percolate obtained from both cans were analyzed for the PO<sub>4</sub> and NO<sub>3</sub> content. The results given below indicate that an excess of both ions was present.

*Parts per million.*

	PO <sub>4</sub>	NO <sub>3</sub>
Can 1.....	55. 7	1, 329
Can 2.....	65. 5	886

The soil in can 85 received only distilled water from May, 1920, to May, 1921, after which tap water was used. Hoagland's nutrient was occasionally added. Soil samples were taken from can 85 on October 8, 1920, and also in September, 1921. A partial analysis of a 1 to 5 aqueous extract of the dried soil gave the following results when expressed in terms of the dried soil:

*Soil samples from can 85, October 8, 1920.*

Parts per million.		Parts per million.	
Ca.....	22	Cl.....	18
Mg.....	9	HCO <sub>3</sub> .....	76
Na+K (calculated as Na).....	3	SO <sub>4</sub> .....	15
Total solids as sulfates.....	128	NO <sub>3</sub> .....	40
SiO <sub>2</sub> .....	19	P <sub>H</sub> of extract (colorimetric).....	6. 6

*Soil samples from can 85, September, 1921.*

Parts per million.		Parts per million.	
Ca.....	18	Cl.....	31
Mg.....	8	HCO <sub>3</sub> .....	61
Na.....	4	SO <sub>4</sub> .....	44
K.....	27	NO <sub>3</sub> .....	22
Total solids.....	313	P <sub>H</sub> of extract (colorimetric).....	6. 6
SiO <sub>2</sub> .....	36		

The trees were removed from cans 1, 2, and 85 on September 20, 1921. Tree 85 was free from mottle-leaf, as were all the trees in the soil series, and was making excellent growth. Plate 5, A, shows the air-dried root systems of trees 1 and 85, grown in sand and in soil respectively. It is evident that starting with trees of nearly similar size, as good growth may be obtained in sand as in soil cultures. The root systems obtained in the sand cultures were somewhat coarser than those obtained in the soil cultures, the root system in the soil cultures being extremely fibrous (Pl. 5, B).

The initial operation in removing a tree from the can was to remove and count the leaves, which were then cleaned with a dry cloth. The shoots were removed at the trunk and after being cut up finely were placed in paper bags. The lid of the can was then removed and soil samples taken. The root system was obtained intact by washing the sand or soil from the tilted can with a strong jet of water. After carefully removing the adhering sand or soil from the rootlets with tap water, they were washed with distilled water. The upper portion of the tree axis was sawed off close to the first lateral root and was designated as the trunk. In cases where the trunk had been previously coated with whitewash it was cleaned with a soft scrubbing brush and tap water and then rinsed with distilled water.

When air dry the trunk was further cleaned with a soft wire brush. All new root laterals formed subsequent to the planting of the tree were removed, placed in bags, and were designated as "rootlets." The root axis, together with any pruned laterals which were present when the tree was first planted in the can, were designated as "root." The root was thoroughly cleaned with the use of a soft wire brush.

The several portions of each tree were dried to constant weight at 60° to 70° C. The rootlets were then shaken in the upper compartment of a set of soil sieves, to remove as much adhering matter as possible. In spite of the care taken in cleaning the rootlets the amount of silica still adhering to them was sufficient to require the calculation of analytical results to a silica-free basis. In the case of trees grown in soil the task of obtaining clean rootlets was so difficult that determinations of ash constituents were omitted from the analyses.

The dry weight, number of leaves, and water transpired for each of the three trees are given in Table II.

TABLE II.—The dry weight of various portions of the trees, the number of leaves, and the water transpired

Tree No.	Number of leaves on tree.	Dry weight (60° to 70° C.).						Total nutrient added.	Total distilled water added.	Total drainage water.	Transpiration.	Total transpiration per total dry weight of trees.
		Leaves.	Shoots.	Trunk.	Root.	Rootlets.	Total.					
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Liters.	Liters.	Liters.	Liters.	
1	996	237.1	122.5	194	212	136	901.6	243	125	139.5	228	0.253
2	302	76.5	20.7	126	64	57	344.2	187	83	173	97	0.282
85	1,042	205.9	96	140.5	135	129.5	706.9	a 27	b 29	0	166	0.235

a After May, 1921.

b May, 1920, to May, 1921. Tap water after May, 1921, 110.2 liters.

When the trees were removed from the cans, their appearance was noted. Tree 1 was an excellent tree with large top and splendid foliage. A few leaves tended to split along the midribs. The root system was large, well developed, and filled practically the entire can.

Tree 2 was the smallest in its series, but was smallest when planted. The leaves were excellent. The lowermost portion of the root axis had died, but a large mass of healthy laterals developed directly above the dead portion. Tree 85 was splendidly developed and was typical of its series. The rootlets were much more finely divided than in the sand series (Pl. 5, A, B).

The dry leaves, shoots, and rootlets were passed through a large hand mill, and any pieces remaining underground were removed. The trunk and root portions were sawed into thin sections, the sawdust being used to represent the trunk and root, respectively.

When ready for analysis the samples were thoroughly mixed and so far as the material available permitted duplicate determinations were run. The methods of analysis employed were essentially those used by Kelley and Cummins (6) in their analyses of citrus material. Manganese was determined colorimetrically by the persulphate method, which is very satisfactory for small amounts (11). Iron was also determined colorimetrically. A few sodium and potassium determinations were made, using the filtrate after the Fe, Al,  $\text{PO}_4$ , Ca and Mg were removed, but the analyses indicated that low results were obtained. Subsequently more satisfactory results were obtained by using the filtrate from the sulphate determination.

The analytical results for the several portions of each tree are presented in Tables III to VII, inclusive. The data have been calculated as percentage of dry matter and as percentage of ash. The figures given are the averages of two closely agreeing duplicates. It is seen that the total percentage of the ash approximates 100 in most cases, but in none reaches it. Those who are familiar with the details of analytical work of this kind appreciate its difficulties. If the reader be one who insists upon total percentages equaling 100 the authors can merely say, in common with other workers in this field, "peccavimus."

TABLE III.—Analyses of leaves

Element.	Expressed as dry matter.			Expressed as ash.		
	Tree 1.	Tree 2.	Tree 85.	Tree 1.	Tree 2.	Tree 85.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
N.....	3. 24	3. 14	2. 15	.....	.....	.....
S.....	0. 31	0. 30	0. 29	.....	.....	.....
P.....	0. 19	0. 20	0. 15	.....	.....	.....
Ash.....	14. 37	14. 32	13. 72	.....	.....	.....
K.....	3. 54	3. 56	1. 44	24. 59	24. 91	10. 51
Na.....	0. 13	0. 21	0. 16	0. 90	1. 43	1. 16
Ca.....	2. 71	2. 66	3. 94	18. 85	18. 57	28. 66
Mg.....	0. 31	0. 26	0. 37	2. 14	1. 75	2. 65
Mn.....	0. 004	0. 004	0. 002	0. 03	0. 03	0. 02
Fe.....	0. 025	0. 04	0. 03	0. 17	0. 26	0. 23
Al.....	0. 17	0. 14	0. 08	1. 14	0. 97	0. 59
Cl.....	0. 04	0. 05	0. 02	0. 26	0. 31	0. 15
$\text{CO}_3$ .....	.....	.....	.....	40. 70	40. 93	42. 64
$\text{SO}_4$ .....	.....	.....	.....	3. 18	3. 17	3. 91
$\text{SiO}_3$ .....	0. 39	0. 43	0. 47	2. 75	3. 02	3. 43
$\text{PO}_4$ .....	.....	.....	.....	3. 92	4. 02	3. 23
Total.....	.....	.....	.....	98. 63	99. 37	97. 18

TABLE IV.—Analyses of shoots

Element.	Expressed as dry matter.			Expressed as ash.		
	Tree 1.	Tree 2.	Tree 85.	Tree 1.	Tree 2.	Tree 85.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
N . . . . .	1.55	1.76	0.79			
S . . . . .						
P . . . . .	0.21	0.23	0.14			
Ash . . . . .	6.81	8.30	5.69			
K . . . . .	1.15	1.22	0.55	16.85	14.47	9.79
Na . . . . .	0.13	0.27	0.08	1.88	3.23	1.47
Ca . . . . .	1.46	1.88	1.58	21.34	22.62	27.71
Mg . . . . .	0.21	0.22	0.18	3.06	2.67	3.19
Mn . . . . .	0.001	0.002	Trace.	0.02	0.02	Trace.
Fe . . . . .	0.02	0.02	0.05	0.32	0.23	0.89
Al . . . . .	0.10	0.10	0.07	1.46	1.15	1.15
Cl . . . . .	0.01	0.01	0.01	0.14	0.11	0.21
CO <sub>3</sub> . . . . .				35.90	39.91	40.95
SO <sub>4</sub> . . . . .				3.13	2.38	3.01
SiO <sub>3</sub> . . . . .	0.11	0.12	0.16	1.53	1.48	2.75
PO <sub>4</sub> . . . . .				8.58	7.64	6.45
Total . . . . .				94.21	95.91	97.57

TABLE V.—Analyses of trunks

Element.	Expressed as dry matter			Expressed as ash.		
	Tree 1.	Tree 2.	Tree 85.	Tree 1.	Tree 2.	Tree 85.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
N . . . . .	0.05	0.15	0.40			
S . . . . .	0.03	0.07	0.03			
P . . . . .	0.09	0.11	0.07			
Ash . . . . .	2.85	3.22	2.68			
K . . . . .	0.39	0.31	0.22	13.57	9.40	10.57
Na . . . . .	0.18	0.24	0.11	6.03	7.45	4.86
Ca . . . . .	0.68	0.80	0.51	23.91	24.77	24.20
Mg . . . . .	0.09	0.09	0.07	3.12	2.77	3.09
Mn . . . . .	Trace.	Trace.	Trace.			
Fe . . . . .	0.003	0.02	0.01	0.11	0.40	0.51
Al . . . . .	0.09	0.16	0.09	3.06	4.92	4.40
Cl . . . . .	Trace.	0.001	0.006		0.04	0.28
CO <sub>3</sub> . . . . .				38.02	34.18	33.45
SO <sub>4</sub> . . . . .				1.96	3.46	4.51
SiO <sub>3</sub> . . . . .	0.04	0.11	0.06	1.31	3.15	2.94
PO <sub>4</sub> . . . . .				5.11	3.70	3.97
Total . . . . .				96.20	94.24	92.87

TABLE VI.—Analyses of roots

Element.	Expressed as dry matter.			Expressed as ash.		
	Tree 1.	Tree 2.	Tree 85.	Tree 1.	Tree 2.	Tree 85.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
N.....	1.01	0.98	0.82			
S.....	0.01	0.13	0.06			
Ash.....	3.03	3.08	2.77			
K.....	0.36	0.33	0.28	11.74	10.55	10.05
Na.....	0.14	0.10	0.07	4.33	3.15	2.42
Ca.....	0.72	0.71	0.70	23.77	23.06	25.26
Mg.....	0.08	0.09	0.05	2.69	2.87	1.74
Mn.....	Trace.	Trace.	Trace.			
Fe.....	0.02	0.02	0.02	0.50	0.49	0.63
Al.....	0.08	0.13	0.07	2.41	4.10	2.35
Cl.....	0.003	0.006	0.016	0.09	0.17	0.59
CO <sub>3</sub> .....				38.28	33.15	41.17
SO <sub>4</sub> .....				2.51	4.47	2.38
SiO <sub>3</sub> .....	0.08	0.14	0.15	2.75	4.68	5.35
PO <sub>4</sub> .....				7.74	9.43	5.55
Total.....				96.81	96.12	97.49

TABLE VII.—Analyses of rootlets calculated to a silica free basis

Element.	Expressed as dry matter.		Expressed as ash.	
	Tree 1.	Tree 2.	Tree 1.	Tree 2.
N.....	2.26	2.22		
S.....	0.29	0.27		
P.....	0.69	0.56		
Ash.....	11.93	11.19		
K.....	2.04	2.38	17.14	21.24
Na.....	0.12	0.09	1.02	0.82
Ca.....	2.36	2.01	19.78	17.98
Mg.....	0.36	0.34	3.06	2.98
Mn.....	0.02	0.02	0.16	0.19
Fe.....	0.04	0.04	0.30	0.28
Al.....	0.36	0.36	3.03	3.22
Cl.....	0.12	0.13	0.89	1.12
CO <sub>3</sub> .....			22.25	24.00
SO <sub>4</sub> .....			8.89	8.46
PO <sub>4</sub> .....			19.65	14.62
Total.....			96.17	94.91

In the analyses reported by Kelley and Cummins (6) the carbonates of the ash were not determined. At their suggestion these determinations have been included in the present studies. With rootlets it is practically impossible to remove all of the adhering sand, and consequently the results obtained for the rootlets (Table VII) have been calculated as percentages on a silica-free basis. The rootlets of tree 85 (soil culture) could not be sufficiently freed from adhering soil to permit of satisfactory analyses.

The precautions necessary in distinguishing between cause and effect, when interpreting analytical data of plant tissue, have already been emphasized (6). In the dry matter there is a progressive decrease in the percentage of total nitrogen in the sand and soil cultures as we pass from the leaves toward the root, where there may be a slight increase which becomes augmented in the rootlets, whose percentage is considerably below that found in the leaves but greater than that found in the shoots.

The percentage of total sulphur was approximately the same for the corresponding parts of the trees in both sand and soil, the shoots and root in every case containing but a trace.

The percentage of total phosphorus was usually somewhat greater for the sand than for the soil cultures. The leaves and shoots contained approximately the same percentage of total phosphorus; while the trunk and roots contained considerably less, with an appreciable increase in the rootlets above that of the roots.

We find the total ash content of the parts examined to be greater in each case for the sand than for the soil culture. The ash of the leaves was approximately double that of the shoots which in turn was more than double that of the trunk and root.

No significant difference as regards total ash existed between the trunk and root. It is of interest to note that the leaves and rootlets contain in their dry matter the greatest percentage of total ash and of nitrogen, which no doubt indicates that here the assimilatory processes are most active.

The percentage of  $\text{CO}_2$  constitutes approximately four-tenths of the total ash, except in the rootlets, where it is only about two-tenths. The large percentage of  $\text{CO}_2$  found in the ash of various parts of the citrus tree may indicate that not only the leaves, but the other portions of the tree as well, contain large quantities of organic acids.

The percentage of  $\text{SiO}_2$  in the ash varies widely in the various parts.

The percentage content of potassium (K) in the ash of citrus leaves obtained in the field (Kelley and Cummins (6)) was approximately 20 per cent when 1 week old, 13 per cent when 6 weeks old, 6 per cent when 6 months to 2 years old, and 2 per cent when 3 or more years old. In the sand cultures the potassium constituted approximately 25 per cent of the total ash of the leaves, about 17 per cent of that of the shoots and about 11 per cent of that of the trunk and roots, respectively. In the soil culture, the potassium in the ash of all parts of the tree examined was approximately 10 per cent. The leaves collected from trees in these cultures probably ranged from 3 to 17 months of age and therefore represent a mixed sample so far as age is concerned. The data for potassium indicate that not only the age of the leaf but also the composition of the medium in which the tree grew may influence the percentage content of potassium in the various parts. The data do not necessarily conflict therefore with those obtained by Kelley and Cummins.

The sodium (Na) content of the ash of the leaves and shoots is relatively small compared with that of potassium. The difficulty of accurately determining a small amount of Na in presence of considerable amounts of K will be appreciated by those familiar with analytical work. The data show that the trunk and root contain a much larger percentage of sodium in the ash than either the leaves or shoots. As the location of the sodium storage in citrus trees is of considerable interest and the data thus far obtained are too meager to justify any far-reaching assumptions,



we will reserve further discussion of the matter until the results of experiments in sand, in which the culture solution contained large amounts of sodium chlorid, have been reported.

The percentages of calcium (Ca) in the ash of the leaves and shoots of the soil culture were higher than for the sand cultures; the trunk and roots contained, approximately, the same percentages respectively in all three cultures. The calcium in the total ash varied from 18 to 30 per cent in the various parts in both sand and soil cultures. The percentage content of calcium in the leaves of the sand cultures was considerably lower than that of the soil culture, while the potassium relation was the reverse of this. These as well as previous analyses have shown that citrus trees have a large capacity for absorbing calcium. Coville with the aid of Breazeale (3) has reported the percentage of calcium oxid in the ash of other trees as compared with that of citrus. They have found that the percentage of CaO in dried freshly fallen leaves ranged from 1.73 per cent in Red Oak (*Quercus rubra*), to 6.77 per cent in orange (*Citrus aurantium*) with 10 other species of forest trees having intermediate values, basswood (*Tilia Americana*) attaining the second highest percentage, or 4.50 per cent.

It is of interest to note the consistently low percentage of magnesium (Mg) in the ash of the various parts of the trees in the sand and soil cultures.

The percentage of aluminum (Al) in the ash of the trunk, root, and rootlets is greater than that of the leaves or shoots. The small percentage of aluminum in the ash of the leaves and shoots indicates that these parts of the trees were thoroughly cleaned from adhering dust. As the nutrient medium used in the sand cultures contained no aluminum, we would expect the trees in these cultures to contain but little.

The percentage of iron (Fe) in the ash of the various parts of the three trees ranged from 0.2 to 0.9 per cent. The percentage of manganese (Mn) was usually very low. The percentage of chlorin (Cl) in the ash ranged usually below 1.2 per cent. The percentage of sulphate ( $\text{SO}_4$ ) present in the ash was considerably higher than that of chlorin, being 2 to 5 per cent, although the rootlets in the sand culture showed over 8 per cent.

The percentage of phosphate ( $\text{PO}_4$ ) in the ash of the shoots was considerably higher in both the sand and soil cultures than that of the leaves, with a decrease in the trunks and an increase in the roots. The ash of the rootlets in the sand cultures contained about 17 per cent phosphate as compared with 8 per cent in the ash of the shoots and 4 per cent in that of the leaves.

It is of interest to note the high percentage content of K, Ca, and  $\text{PO}_4$  in the ash of the rootlets in the sand culture, constituting over 50 per cent of the ash of the rootlets, with a marked reduction in the percentage of  $\text{CO}_2$ . The percentage of silica-free ash in the rootlets approaches that in the leaves and is much greater than that in the shoots, trunk, or root.

The percentage of  $\text{PO}_4$  in the ash of rootlets was greater than for any other portion of the tree. The largest percentages of mineral constituents appear to be deposited in those parts of the tree in which the assimilatory processes are most active.

The sap of citrus leaves possesses the power to neutralize considerable base. Kelley and Cummins (6) have shown that 10 cc. of normal leaf sap required 3 cc. of N/10 alkali for neutralization, while mottle-leaf

sap required 7.05 cc. of N/10 alkali. Protein formation or decomposition may bring about the formation of organic acids (8, p. 237-249). Since a large portion of the calcium in many plants is used for the neutralization of acids, there should be more calcium in plants producing large amounts of acid. The analyses indicate that this may be the case with orange trees, namely, that one of the chief functions of calcium in the tree may be the neutralization of the organic acids. Opportunity to test out this hypothesis further will be afforded in cultures now growing.

#### SUMMARY

(1) Data have been presented to show that the nutrition of young trees may be studied in a quantitative way under controlled conditions by the procedure described.

(2) The reaction of the nutrient solution used shifted from  $P_H$  5.2 to 5.9 when the solution was in contact with sand, and further toward the neutral point when in contact with sand containing citrus rootlets.

(3) The relative amounts of new growth produced by the trees grown in sand or soil cultures were quite similar when one estimates the ratio between total dry weight and the dry weight of corresponding portions. The ratio of total transpiration to total dry weight of tree was approximately the same in both cases. The rootlets of the trees grown in soil were much more finely divided than those grown in sand.

(4) Analyses of the trees showed both a high calcium (Ca) content and a high Ca/N ratio.

(5) The percentages of total phosphorus in leaves and shoots were approximately equal.

(6) The leaves and rootlets contain in their dry matter the greatest percentage of total ash and of nitrogen.

(7) The ash obtained from all parts of the tree contained large amounts of carbonate, although the rootlets contained less than the other portions.

(8) The ash of all parts of the trees was markedly rich in potassium and relatively poor in sodium. The former was relatively uniform in the various parts of the tree, but the distribution of the latter was more variable.

(9) The various parts of the trees likewise contained large amounts of calcium and relatively small amounts of magnesium. Both constituents show fairly uniform distribution throughout the tree.

(10) The distribution of sulphate in the ash of the various parts of the tree is quite uniform with the exception that the rootlets contained nearly three times as much as the other portions of the tree.

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PLATE 1

A.—A typical trench with cans of sand containing orange trees just beginning growth.

B.—Showing attachment of air-suction hose to one of a series of drainage bottles. The drainage tube is removed from the drainage pipe. Can and bottle are raised considerably for photographic purposes.

C.—Device for weighing cans.

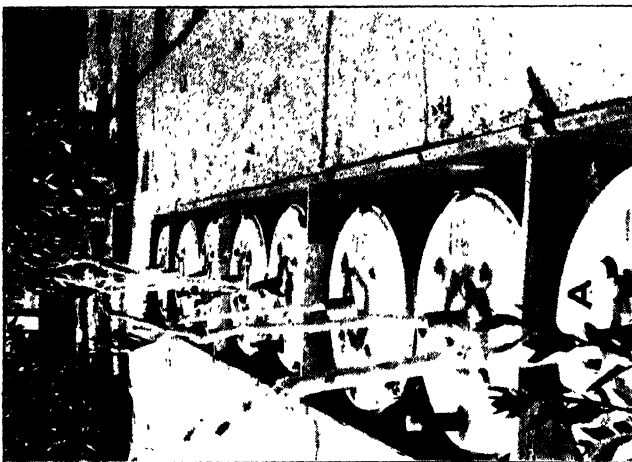
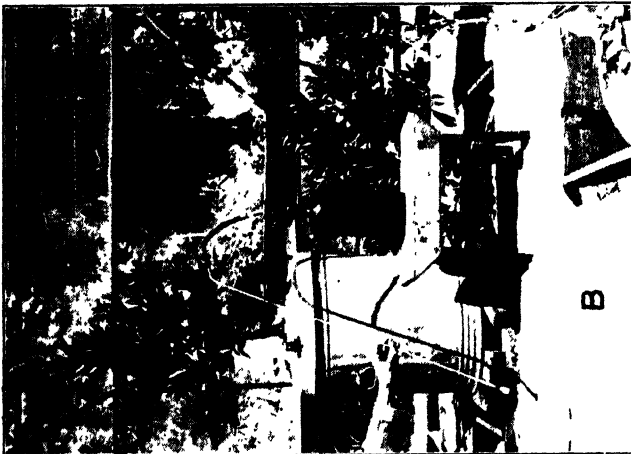
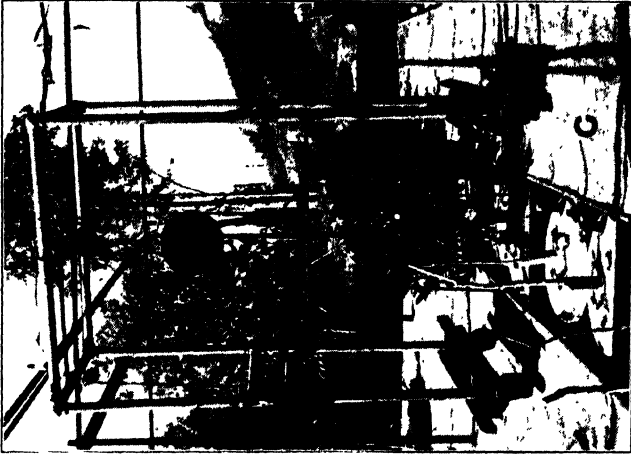




PLATE 2

A.—Walnut tree after six months in covered tank of sand that receives a nutrient solution made up with distilled water.

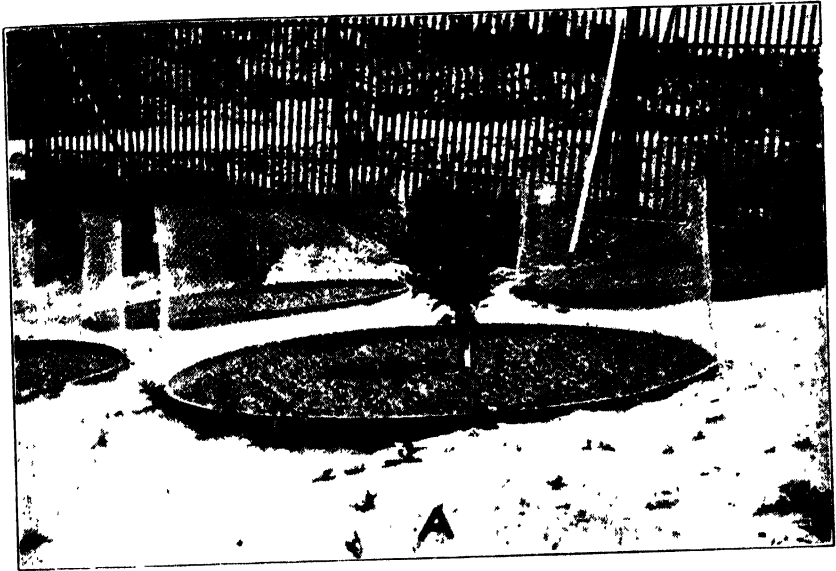
B.—Valencia orange trees after 15 months in cans of sand to which Hoagland's nutrient has been added.



**PLATE 3**

**A.—Valencia orange tree in large tank of soil. Seven feet 8 inches in diameter and 4 feet 4 inches in depth.**

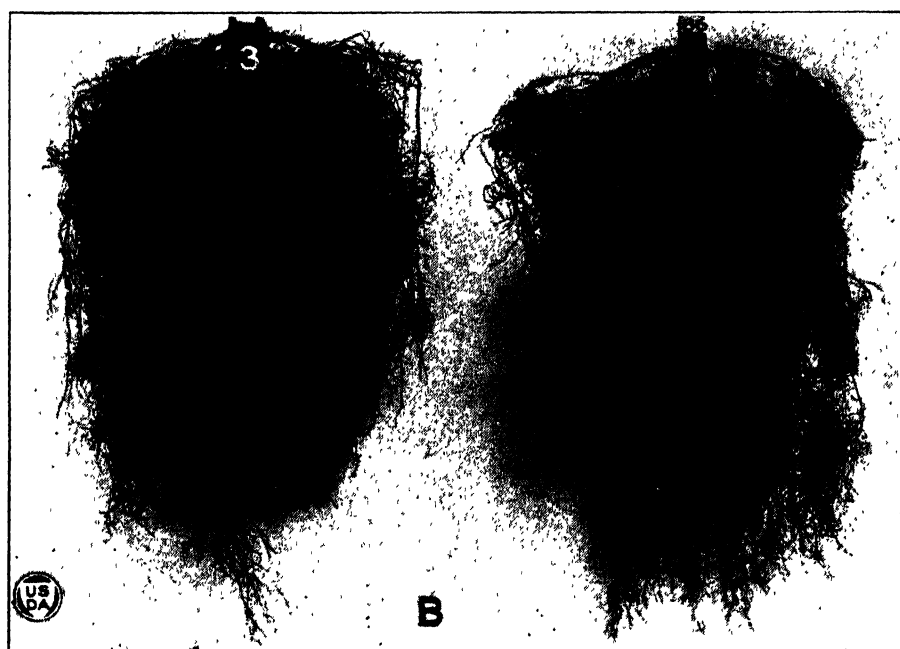
**B.—Growth typical of trees in soil series 84-88 receiving Hoagland's nutrient at intervals.**



**PLATE 5**

**A.**—Air-dried root systems of trees 1 and 85, in sand and soil, respectively, receiving Hoagland's nutrient solution.

**B.**—Root systems of trees 3 and 86, in sand and soil, respectively, receiving Hoagland's nutrient solution. Note the more finely divided root systems in the soil culture.





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## FURTHER STUDIES OF THE INHERITANCE OF "ROGUE" TYPE IN GARDEN PEAS (*PISUM SATIVUM* L.)<sup>1</sup>

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### INTRODUCTION

Since the rediscovery of Mendel's classical paper on heredity, characters of the garden pea (*Pisum sativum*) have been used to illustrate typical Mendelian inheritance. In 1915 Bateson and Pellew (1) <sup>2</sup> published an account of the genetics of types of peas known as "rogues"—types in which heredity has thus far appeared to be non-Mendelian. This paper was followed by later publications (2, 3) dealing especially with variations in the proportion of rogues obtained from seeds from different parts of the same intergrading intermediate plant. Briefly stated, the facts reported were as follows:

(a) Certain varieties of the garden pea, such as Early Giant, Ne Plus Ultra, and Duke of Albany, are characterized by broad, wavy stipules and leaflets, both having rounded, emarginate apices and broad, straight pods. Occasionally there occur in pure-lines of such varieties plants called "rogues" that are described as "wild" or "vetch-like." The rogues differ from the parent form mainly in the reduction in width of the foliar parts (stipules and leaflets) and of the pods. The stipules of the rogues are narrow and pointed; the pods are narrow and curved along the upper suture. Rogues are not produced with any definite regularity or in such numbers that a characteristic ratio can be established between the rogues and parent form.

(b) Between the parent types and the vetch-like rogue are found intermediate forms, especially in the variety Early Giant. The intermediates are of several classes and may grade almost insensibly into types or rogues. The most usual form is characterized by broad foliage in the early stages of development, but as the plant matures the stipules become pointed and more roguelike. Such a plant is called by Bateson and Pellew an intergrading intermediate. Genetically there are two kinds of intermediates: (1) Those whose progeny consists of many rogues and few intermediates; and (2) those whose progeny consists of few rogues and many intermediates. The rogues from both kinds of families breed true, while the intermediates tend to produce rogues and intermediates in proportions comparable to those of their parents, i. e., there are high and low rogue-producing strains. Nevertheless, no regular ratio of rogues to intermediates can be found in successive generations.

<sup>1</sup> Accepted for publication Jan. 18, 1923. This contribution records cooperative work between the Bureau of Plant Industry and the University of Michigan which was carried on at the Department of Botany, University of Michigan.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 852.

(c) Any zygote derived from the fusion of a type gamete with a rogue gamete, whichever way the cross is made, produces a rogue; an intermediate gamete on union with a type gamete produces an intermediate or nonrogue form.

(d) Rogues are apparently produced more often from the upper (roguelike) portion of the intermediate plants than from the lower (typelike) part. It is also found that pollen grains produce a greater ratio of rogue gametes to nonrogue gametes than do the ovules, and that the proportion of rogue gametes produced by the pollen grains increases more rapidly, in going from the lower to upper flowers, than the proportion of rogue gametes produced by ovules.

(e) Intergrading intermediates of strains not throwing many rogues were found to produce rogues no more often from pods of the upper parts of the plant than from the lower nodes.

(f) Rogues with narrow, pointed stipules and narrow, curved pods, from whatever source, breed true.

(g) The cross rogue  $\times$  type, no matter how made, produces only rogues in the  $F_1$  and succeeding generations. In the seedling stage of the  $F_1$  hybrid until the seventh or eighth node is developed, many of the plants are typelike in the character of the stipules and leaflets. These plants at maturity, with few exceptions, are all rogues. The plants in the  $F_2$  generation are recognizable as rogues at all stages of development. There is apparently no segregation in the  $F_1$  hybrids, at sporogenesis, of the rogue and type factors.

(h) Intervarietal crosses of rogue with type plants of varieties producing rogues give results similar to crosses of rogue with the variety from which the rogue originated as far as the rogue characters are concerned. Side by side with the apparently non-Mendelian behavior of the rogue characters is found the expected Mendelian segregation of such characters as pod shape, color of seed, shape of seed, and height of plant.

Bateson and Pellew (1) have suggested in explanation of the anomalous genetic behavior of the rogue characters that they are due to somatic segregation of the type "elements" in the  $F_1$  hybrid so that the type "elements" are missing from the germ plasm at the time of gamete formation. Intergrading intermediates are assumed to be mosaics of type and rogue tissue, rogue gametes being formed from rogue tissue and type gametes from type tissue.

The present paper is in part a confirmation of the above results. However, a different interpretation of the results is advanced. In addition, data are submitted of the  $F_1$ ,  $F_2$ , and  $F_3$  generations of intervarietal crosses of rogue and type plants with type plants of varieties that apparently do not produce rogues. These crosses have given entirely new results, from which important conclusions have been drawn.

#### EXPERIMENTAL METHOD

The experimental methods used in the work here reported were the same as those described in a previous paper (4). At that time the fact of cross-pollination of rogue flowers by bumblebees was noted. In growing large cultures of peas it is impossible to protect all the individual flowers with paper or cloth coverings. In 1921 an attempt was made to grow peas under wire mosquito netting. This method is too expensive to be used on a large scale, so that in the future it seems advisable to carry on as much as possible of the breeding work with rogues in the greenhouse during the winter.

The results of 1919 and 1920 may be used to illustrate the amount of volunteer crossing that may be expected in the field in crosses between Gradus and Rogue. The number of volunteer hybrids is sufficiently great to make necessary some sort of protection of the rogue plants.

In 1919, 2,932  $F_2$  plants of the crosses *Gradus* rogue with *Gradus* (Table II) were grown. Of the 2,932 plants, 65, or 2.3 per cent, were volunteer crosses made by insects in 1918. The out-crosses were detected by the appearance of such dominant Mendelian characters as smooth seed, blunt pods, and colored flowers in  $F_2$  cultures that should have contained only recessives for the three characters mentioned. Any crosses inter se, or out-crosses with recessive strains, could not be detected, but, apart from a few plants of Early Giant and of Peter Pan, no varieties which did not exhibit one of the three dominant characters were planted in the experimental garden in 1918. In 1920, 3,475  $F_2$  and  $F_3$  plants of the cross rogue with type (Tables II, III and IV) were raised. Among them were 42 plants of obviously foreign origin, or about 1.2 per cent, showing that the amount of volunteer crossing was considerably less in 1919 than in 1918.

Occasional volunteer crosses have been found among the rogues and intermediate rogues, but no count has been kept. However, only one case of cross-pollination by insects has been noted among the thousands of typical *Gradus* plants grown.

Plants used in crosses have been previously inbred for at least one generation. The rogues used were either picked from commercial plantings of *Gradus* or arose de novo in pure cultures of *Gradus* plants. In either case the rogue strains were identical and gave the same results in crosses.

Uncontrolled cross-pollination in *Pisum* on the scale reported here was unlooked for at the beginning of these experiments and no steps were taken to insure self-pollination. By the time it was realized that rogues were an exception to the general rule that self-pollination is invariable in *Pisum* it was too late to protect the hybrid rogue plants in 1919. Aside from the writer's own experience with self-pollinating varieties of peas, his garden technic was of course influenced by the general conclusion of geneticists and practical horticulturists that peas are normally self-pollinated. White (15) found no cross with peas having yellow cotyledons among 10,000 seeds with green cotyledons grown at the Brooklyn botanic garden.

#### BEHAVIOR OF GRADUS ROGUES WHEN CROSSED WITHIN THE GRADUS STRAIN

##### $F_1$ GENERATION OF CROSSES BETWEEN GRADUS AND GRADUS ROGUE

In a former publication (4) the results in the  $F_1$  generation of crossing typical *Gradus* with *Gradus* rogue<sup>3</sup> are reported.

The  $F_1$  seedlings were variable in regard to stipule shape, showing gradations from typelike to roguelike plants. At maturity with one exception the  $F_1$  plants were all rogues.

In addition to the hybrids between rogue and type previously listed by the author (4), 134  $F_1$  plants from 37 other crosses were grown, making a total from all sources of 282  $F_1$  plants of the cross *Gradus* × *Gradus* rogue, and reciprocal. Of the 282 plants all but three were classified as rogues at maturity.

<sup>3</sup> The name "*Gradus* rogue" or "Rogue" as used throughout this paper refers to the same type of mutation as that previously referred to (4) as a "Rabbit-ear" Rogue.



Two of the exceptional plants occurred in a single  $F_1$  culture,<sup>4</sup> of which two plants were thorough rogues, while two others were intergrading intermediates, i. e., they were typelike at the lower nodes, but at upper nodes the stipules were pointed but much broader than in typical rogues. In the  $F_2$  generation the progeny of the intergrading intermediates consisted of 32 and 33 rogues, respectively.

The third exceptional plant among the  $F_1$  of rogue  $\times$  type was described in 1919 (4). Its offspring in 1919 consisted of 19 intergrading intermediates and 15 type plants. The same cross and its reciprocal produced 7 other  $F_1$  plants, described as rogues at maturity. From the character of the  $F_2$  progenies (Table I) it is probable that the  $F_1$  plants were in reality extreme intergrading intermediates, as the  $F_2$  is characteristic of the progeny of intergrading intermediates. The plants behaved as intergrading intermediates of the sort described by Bateson and Pellew (1) as producing types, intergrading intermediates, and few or no rogues.

TABLE I.— $F_1$  and  $F_2$  generation of atypical hybrids of *Gradus rogue*  $\times$  *Gradus type* and reciprocal

1919 culture No.	Pedigree	$F_2$		
		Rogues.	Intergrading intermediates.	Types.
9.1207	(G13-1-6-1t $\times$ G19-1-3r)—1.....	0	27	0
9.1208	(G13-1-6-1t $\times$ G19-1-3r)—2.....	0	23	0
9.1209	(G13-1-6-1t $\times$ G19-1-3r)—3.....	0	9	2
9.1210	(G13-1-6-1t $\times$ G19-1-3r)—4.....	0	0	23
9.1216	(G19-1-3r $\times$ G13-1-6-1t)—1.....	2	8	3
9.1217	(G19-1-3r $\times$ G13-1-6-1t)—2.....	0	27	0
9.1218	(G19-1-3r $\times$ G13-1-6-1t)—3.....	0	0	4
9.1219	(G19-1-3r $\times$ G13-1-6-1t)—4.. Type foliage....	0	19	15

#### $F_2$ AND $F_3$ GENERATIONS OF CROSSES BETWEEN GRADUS AND GRADUS ROGUE

From 184  $F_1$  hybrids of *Gradus*  $\times$  *Gradus rogue* and reciprocal 4,319  $F_2$  plants were raised in 1919 and 1920. In the seedling stage the greater portion were easily recognizable as rogues; others could be termed intermediates, but none showed the typelike appearance found among the  $F_1$  seedlings. (Among the 4,319 plants shown in Table II, 81, or 1.8 per cent, were from foreign pollination, as explained elsewhere in this paper. The volunteer out-crosses were generally distinguished in the seedling stage as plants with typelike stipules.) At maturity the plants were either rogues or various sorts of intermediates. Thirty-nine  $F_2$  rogues gave in the  $F_3$  generation 575 plants, all rogues except five plants (0.8 per cent). The products of volunteer out-crossing in 1919 are shown in Table III.

<sup>4</sup> The intergrading intermediates were plants No. 3 and No. 4 of cross (G10-1-12-1t  $\times$  G23-1-1-16r) in the  $F_1$  generation.

TABLE II <sup>a</sup>.—*Gradus rogue* × *Gradus type* and reciprocal in the *F*<sub>2</sub> generation

Culture No.	Rogues.	Crosses.	Culture No.	Rogues.	Crosses.
1919:			1919—Continued.		
9.1200.....	10		9.1307.....	29	
9.1201.....	3		9.1308.....	18	
9.1202.....	13		9.1309.....	25	
9.1203.....	16		9.1310.....	58	2
9.1204.....	4		9.1317.....	13	3
9.1205.....	10		9.1318.....	44	
9.1206.....	3		9.1319.....	73	
9.1211.....	29		9.1320.....	108	4
9.1212.....	39		9.1321.....	31	
9.1213.....	36		9.1322.....	43	
9.1214.....	35		9.1323.....	4	
9.1215.....	54	2	9.1324.....	21	
9.1220.....	4		9.1325.....	25	
9.1221.....	4		9.1326.....	46	
9.1222.....	2				
9.1223.....	2		Total.....	2,867	65
9.1226.....	12				
9.1227.....	14		1920:		
9.1251.....	80		0.89.....	15	2
9.1252.....	82		0.90.....	17	1
9.1253.....	86		0.91.....	12	
9.1254.....	10		0.92.....	37	1
9.1255.....	208		0.93.....	12	
9.1265.....	37	3	0.94.....	7	
9.1266.....	27		0.95.....	15	1
9.1267.....	24		0.96.....	7	
9.1268.....	51	1	0.97.....	30	
9.1269.....	15	1	0.98.....	48	1
9.1270.....	37		0.99.....	10	
9.1271.....	39		0.100.....	6	
9.1272.....	47		0.101.....	14	
9.1273.....	28		0.102.....	7	
9.1274.....	27	3	0.103.....	15	
9.1275.....	34	4	0.104.....	14	1
9.1276.....	57	3	0.105.....	14	
9.1277.....	54		0.106.....	12	
9.1278.....	43	1	0.107.....	30	
9.1279.....	6		0.108.....	26	
9.1280.....	19		0.109.....	10	
9.1284.....	118	4	0.110.....	9	
9.1285.....	73	1	0.112.....	21	2
9.1286.....	36		0.113.....	20	1
9.1287.....	49	1	0.114.....	13	
9.1288.....	85	2	0.115.....	8	
9.1289.....	22	1	0.116.....	18	
9.1293.....	6	1	0.117.....	18	
9.1294.....	52	4	0.118.....	15	
9.1295.....	73	12	0.119.....	20	
9.1296.....	54		0.120.....	22	
9.1297.....	19		0.121.....	21	
9.1298.....	17	2	0.122.....	18	
9.1299.....	43	1	0.123.....	31	
9.1300.....	32		0.124.....	12	
9.1301.....	95		0.125.....	25	
9.1302.....	77	7	0.126.....	14	
9.1304.....	45	1	0.127.....	25	
9.1303.....	15		0.128.....	22	
9.1305.....	71	1	0.129.....	16	
9.1306.....	46		0.130.....	15	

<sup>a</sup> In Tables II, III, and IV, unless otherwise noted, the parent plants were rogues in each instance. The column headed "Crosses" shows the number of volunteer hybrids occurring in the various cultures.

TABLE II.—*Gradus rogue* X *Gradus* type and reciprocal in the  $F_2$  generation—Continued.

Culture No.	Rogues.	Crosses.	Culture No.	Rogues.	Crosses.
1920—Continued.			1920—Continued.		
o.131.....	36		o.199.....	9	I
o.184.....			o.200.....	32	
o.185.....			o.202.....	6	
o.186.....	23		o.203.....	15	
o.187.....	38		o.204.....	18	
o.188.....	37		o.205.....	30	
o.189.....	34		o.206.....	37	2
o.190.....	8		o.207.....	3	
o.191.....	26	2	o.208.....	14	
o.192.....	10		o.209.....	39	
o.193.....	26		o.210.....	36	
o.194.....	21		o.211.....	7	
o.195.....	31		o.212.....	2	
o.196.....	29		o.213.....	32	
o.197.....	7				
o.198.....	21	I	Total (1919-20).....	4,238	81

TABLE III.—*Gradus rogue* X *Gradus* type and reciprocal in the  $F_3$  generation

Culture No.	Rogues.	Crosses.	Culture No.	Rogues.	Crosses.
1920:			1920—Continued.		
o.371.....	22		o.391.....	10	
o.372.....	10		o.392.....	15	
o.373.....	23		o.393.....	12	
o.374.....	12		o.394.....	14	
o.375.....	11		o.395.....	16	
o.376.....	13		o.396.....	24	2
o.377.....	16		o.397.....	3	
o.378.....	13		o.398.....	25	
o.379.....	3		o.399.....	17	I
o.380.....	6		o.400.....	21	
o.381.....	6		o.401.....	18	
o.382.....	15		o.402.....	11	
o.383.....	10		o.403.....	22	
o.384.....	20		o.404.....	17	
o.385.....	12		o.405.....	18	
o.386.....	13		o.406.....	23	
o.387.....	17		o.407.....	23	2
o.388.....	17		o.408.....	18	
o.389.....	15				
o.390.....	14		Total.....	575	5

# $F_1$ AND $F_2$ GENERATIONS OF CROSSES BETWEEN *GRADUS* TYPE AND THE $F_1$ OF *GRADUS* TYPE WITH *GRADUS* ROGUE

The cross type X (type X rogue) gave an  $F_1$  of 88 plants which showed a wide range of variation from typelike to roguelike plants in the seedling stage, but which at maturity were all rogues. Sixty-six  $F_1$  plants of the sesquireciprocal crosses gave an  $F_2$  progeny (Table IV) of 1,477 rogues, 6 intermediate types, and 22 volunteer hybrids (1.4 per cent).

The exceptional intermediate types occurred in the  $F_2$  culture 0.322, which also contained 23 rogues and 1 out-crossed plant. Seven other families from the original cross which produced culture 0.322 gave only rogues in the  $F_2$  generation.

TABLE IV.—Back-crosses *Gradus type* × (*Gradus type* × *Gradus rogue*) in  $F_2$  generation

Culture No.	Rogues.	Others.	Crosses.	Culture No.	Rogues.	Others.	Crosses
1920:				1920—Contd.			
0.216.....	3			0.325.....	23		
0.217.....	2			0.326.....	16		1
0.292.....	36		2	0.327.....	22		
0.293.....	25			0.328.....	17		
0.294.....	23			0.329.....	40		
0.295.....	23			0.330.....	31		
0.296.....	2			0.331.....	41		
0.297.....	31			0.333.....	18		
0.298.....	18			0.334.....	54		1
0.299.....	11			0.335.....	22		
0.300.....	11			0.336.....	36		
0.301.....	38			0.338.....	73		
0.302.....	12			0.341.....	18		1
0.303.....	27			0.342.....	21		
0.304.....	1			0.343.....	20		2
0.305.....	6			0.344.....	6		
0.306.....	27			0.345.....	28		
0.307.....	17			0.346.....	20		
0.308.....	20		1	0.347.....	15		
0.309.....	20			0.348.....	23		
0.310.....	16			0.349.....	18		
0.311.....	44		4	0.350.....	23		
0.312.....	33			0.351.....	15		
0.313.....	17			0.352.....	30		
0.314.....	10			0.353.....	26		
0.315.....	44			0.355.....	7		
0.316.....	45		2	0.356.....	13		
0.317.....	38		2	0.357.....	18		5
0.318.....	7			0.358.....	27		
0.319.....	16			0.368.....	14		
0.320.....	31			0.369.....	22		
0.321.....	3			0.370.....	15		
0.322.....	23	6	1	Total...	1,487	6	22
0.324.....	35						

#### STATISTICAL STUDY OF THE INHERITANCE OF STIPULE SHAPE IN GRADUS ROGUE AND GRADUS TYPE

In order to get a more exact comparison of the rogues and types, on the one hand, and the  $F_1$  and  $F_2$  generations of hybrids between the two, measurements of the length and width of the stipules from the fourth to the ninth node were made in the various categories of plants. The ratio of width to length of stipule was then calculated. Because of disease and mechanical injury the same number of stipules could not be measured at each node in each group of plants. The class frequency distributions (Table V) are, therefore, expressed as percentages of the total number of variates. In this manner 49 pure type plants, 43 pure rogues, 69  $F_1$  hybrids, and 104  $F_2$  plants were measured.

The mean, standard deviation, and coefficient of variation of the ratios at each node and for the plant as a whole were calculated from the data in Table V. These statistical constants are given in Table VI. Figure 1 shows the graphs obtained by plotting the means for each node in each

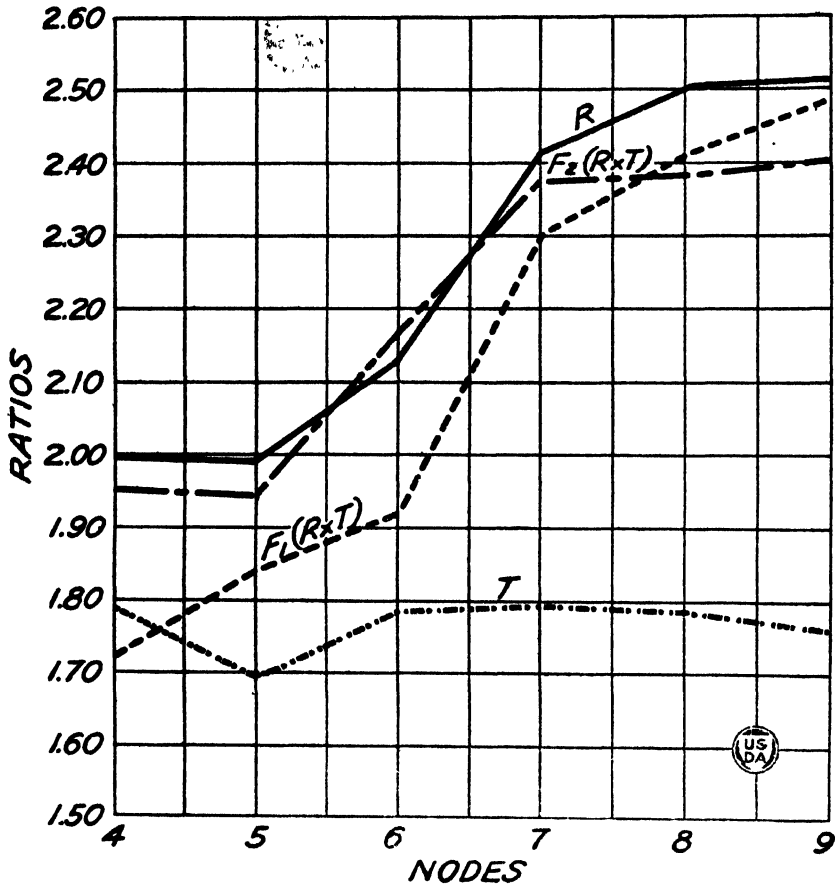


FIG. 1.—Graphs obtained by plotting the mean ratio of  $\frac{\text{length of stipule}}{\text{width of stipule}}$  at the fourth to ninth nodes of Gradus type (T), Rogue (R), and the F<sub>1</sub> and F<sub>2</sub> hybrids of crosses between Gradus and Gradus rogue, based on data from Table VI.

group of plants. The similarity of the F<sub>1</sub> and F<sub>2</sub> generations to the rogue parent is easily seen by inspection of Figure 1 and Tables V and VI. The F<sub>1</sub> hybrids up to the seventh node are like the type, or intermediate between the type and rogue; above the seventh node the plants become rapidly more roguelike. At all nodes the F<sub>1</sub> plants appear more variable than Gradus and are like the rogues in this respect.

TABLE V.—Frequency distribution of ratio  $\left(\frac{\text{length of stipule}}{\text{width of stipule}}\right)$  at the fourth to the ninth nodes in *Gradus* rogue, *Gradus*,  $F_1$  and  $F_2$  hybrid plants. As the number of stipules measured at each node was not always the same, the ratios in any one class are expressed in percentages

Ratio.	Nodes.						
	Fourth.	Fifth.	Sixth.	Seventh.	Eighth.	Ninth.	Total.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
<b>Gradus rogue:</b>							
1.25.....							
1.35.....			3.02	3.02			0.45
1.45.....	2.66	1.20		0			.67
1.55.....	5.32	2.40	1.16	0			1.56
1.65.....	10.64	6.00	1.16	1.51			3.36
1.75.....	13.30	8.40	3.48	0		3.56	4.93
1.85.....	9.31	18.00	9.28	0	3.75	3.56	7.84
1.95.....	5.32	12.00	10.44	3.02	0	1.78	5.79
2.05.....	11.97	25.20	24.36	4.53	2.50	0	12.55
2.15.....	10.64	8.40	10.16	4.53	2.50	0	6.73
2.25.....	15.96	8.40	16.24	10.35	6.25	5.34	10.76
2.35.....	6.65	4.80	10.16	12.08	16.25	5.34	9.64
2.45.....	5.32	3.60	.48	21.14	12.50	1.24	9.42
2.55.....	1.33	1.20	4.64	16.61	21.25	28.44	11.21
2.65.....	0		1.16	19.63	16.25	19.58	8.52
2.75.....	1.33		0	3.02	10.00	8.90	3.58
2.85.....			1.16		0	5.34	.89
2.95.....					6.25	1.78	1.34
3.05.....					1.25	1.78	.45
3.15.....					1.25		.22
	a 75	a 83	a 86	a 66	a 80	a 56	
<b>Gradus:</b>							
1.25.....							
1.35.....	5.40	1.08	2.24				1.59
1.45.....	6.48	3.24	3.36	6.18	1.08		3.78
1.55.....	10.80	19.44	8.96	10.30	7.56	9.52	11.35
1.65.....	11.88	24.84	11.20	13.39	15.12	21.42	15.93
1.75.....	19.44	23.76	30.24	19.57	29.16	33.32	25.30
1.85.....	35.64	18.36	20.16	23.69	27.00	19.04	24.70
1.95.....	3.24	7.56	13.44	13.39	16.20	10.90	10.95
2.05.....	4.32	0	6.72	7.21	2.16	4.76	4.18
2.15.....	0	1.08	3.36	6.18	0	0	.20
2.25.....	0	0	0	0	0	0	0
2.35.....	1.08						.02
2.45.....							
	a 91	a 92	a 89	a 97	a 91	a 42	
<b><math>F_1</math> hybrid:</b>							
1.25.....	.85		1.46				.41
1.35.....	.85	.75	0	.77			.41
1.45.....	5.10	1.50	1.46	0			1.38
1.55.....	16.15	10.50	1.46	.77			4.98
1.65.....	30.60	16.50	10.22	.77			10.11
1.75.....	22.10	16.50	10.95	.77	1.60		9.14
1.85.....	22.10	19.50	20.44	4.62	1.60		12.18
1.95.....	4.25	10.50	13.14	5.39	1.60		6.37
2.05.....	3.40	18.75	12.41	10.01	8.80	1.35	8.44
2.15.....	0	12.00	9.49	11.55	3.20	1.35	5.67
2.25.....	.85	9.00	10.95	13.86	12.00	10.80	8.72
2.35.....	1.70	.75	3.65	17.71	12.00	20.25	8.44
2.45.....		0	.73	8.47	17.60	18.90	6.65
2.55.....		0	2.19	14.63	19.20	24.30	8.86
2.65.....		.75	.73	6.16	10.40	14.85	4.71
2.75.....				2.31	7.20	5.40	2.21
2.85.....				1.54	.80	1.35	.55
2.95.....					3.20	1.35	.69
	a 117	a 132	a 136	a 129	a 124	a 74	

TABLE V.—Frequency distribution of ratio  $\left(\frac{\text{length of stipule}}{\text{width of stipule}}\right)$  at the fourth to the ninth nodes in *Gradus rogue*, *Gradus F<sub>1</sub>*, and *F<sub>2</sub>* hybrid plants. As the number of stipules measured at each node was not always the same, the ratios in any one class are expressed in percentages—Continued.

Ratio.	Nodes.						
	Fourth.	Fifth.	Sixth.	Seventh.	Eighth.	Ninth.	Total.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
<b>F<sub>2</sub> hybrid:</b>							
1.05	.49						.08
1.15	.96	.49					.24
1.25	.96	0					.16
1.35	.96	.49					.24
1.45	.48	.49					.16
1.55	1.45	.98					.41
1.65	6.26	3.42					1.62
1.75	11.20	14.20	.96				4.37
1.85	15.80	22.42	4.33	1.44	.49	.49	7.52
1.95	14.50	20.42	3.85	1.44	.49	.49	6.87
2.05	26.10	21.40	27.40	4.82	4.90	2.94	14.63
2.15	8.20	6.34	21.61	8.18	8.78	5.88	9.70
2.25	8.67	3.42	11.52	16.33	17.55	15.70	13.90
2.35	1.45	3.90	5.30	26.40	20.00	25.00	14.70
2.45	.96	.49	2.88	14.90	17.55	22.10	10.20
2.55	.48	.49	.48	18.20	21.92	13.20	9.55
2.65	.96	0		4.81	4.39	10.30	3.48
2.75	.48	.49		2.40	3.42	2.40	1.53
2.85				.96	.49	1.47	.48
	<sup>a</sup> 207	<sup>a</sup> 205	<sup>a</sup> 208	<sup>a</sup> 208	<sup>a</sup> 205	<sup>a</sup> 204	.....

<sup>a</sup> The actual number of measurements from which the frequency distributions (expressed in percentages) were derived.

TABLE VI.—Constants for stipule shape calculated for each node separately and for the plant as a whole, based on data in Table V

	Nodes.						
	Fourth.	Fifth.	Sixth.	Seventh.	Eighth.	Ninth.	All.
<b>Type:</b>							
M	1.705	1.706	1.782	1.797	1.782	1.766	1.765
$\sigma$	.205	.155	.170	.177	.130	.130	1.53
C. V.	12.05	8.11	9.55	9.88	7.30	7.32	8.66
<b>Rogue:</b>							
M	2.001	1.994	2.133	2.411	2.500	2.515	2.259
$\sigma$	.294	.223	.223	.288	.257	.266	.339
C. V.	14.70	11.20	10.46	11.53	10.28	10.59	15.00
<b>F<sub>1</sub> hybrid:</b>							
M	1.725	1.846	1.926	2.308	2.410	2.485	2.118
$\sigma$	.174	.217	.275	.266	.261	.172	.377
C. V.	10.11	11.79	14.32	11.56	10.82	6.93	17.79
<b>F<sub>2</sub> hybrid:</b>							
M	1.957	1.941	2.173	2.370	2.384	2.405	2.205
$\sigma$	.256	.206	.148	.184	.170	.171	.275
C. V.	13.08	10.61	6.81	7.75	7.13	7.71	12.47

The  $F_2$  hybrids differ from the  $F_1$  generation in being remarkably like the pure rogues at all stages in their development. The stipules at the fourth, fifth, and sixth nodes of the former are especially different from those of the  $F_1$  plants. The measurements serve to emphasize what is plainly seen by inspection of the cultures, namely, that the  $F_2$  seedlings resemble the pure rogues of the same age rather than the young  $F_1$  plants which so generally exhibit a typelike character. To the eye, neither of the hybrid generations, at maturity, differs in any appreciable way from the rogue parent.

Besides measuring the stipules at the fourth to the ninth nodes in the case of the rogue, type, and  $F_1$  and  $F_2$  hybrids, measurements were made of four stipules per plant from about the eighth to the fourteenth node of mature individuals in each category. The mature plants measured included those used in the comparison by nodes of the four groups, and, in the case of all but the  $F_1$  hybrids, additional plants were measured.

In Table VII are shown the frequency distributions and statistical constants calculated for each of the four categories of plants. The range of variation is strikingly significant, the range of the type not overlapping that of the rogue. The  $F_1$  and  $F_2$  hybrids have a range of variation equal in extent to that of the rogue parent. The  $F_2$  generation differs from the pure rogue and the  $F_1$  in that it is less variable as indicated by the coefficients of variation (Coefficient Variation):  $F_2$  generation,  $5.84 \pm 0.2677$ ; rogue,  $7.06 \pm 0.2912$ ;  $F_1$  generation,  $7.09 \pm 0.3662$ , respectively. The probable errors of the differences between the coefficients of variation of the pure rogue and of the  $F_2$ , and of the  $F_2$  and  $F_1$ , are practically three times the difference, in each case indicating that the differences are real. However, the differences in variability of the three groups as shown by the stipule measurements of the mature plant were not apparent on inspection of the cultures.

The resemblance of the  $F_1$  and  $F_2$  hybrids to the pure rogues is further accentuated by a comparison of the mean stipule ratio of the three. The mean for the rogue parent is  $2.339 \pm 0.0096$ ; for the  $F_1$ ,  $2.383 \pm 0.0196$ ; and for the  $F_2$  it is  $2.360 \pm 0.0089$ . There is no significant difference between any of the three means.

Thus in regard to variability, range of variation, and mean stipule ratio, the two hybrid generations resemble only the rogue parent. The influence of the type parent, in crosses between type and rogue is apparent only in the  $F_1$  plant and at only the lower nodes.

#### CROSSES BETWEEN THE GRADUS STRAIN AND MUMMY, A NONROGUE-PRODUCING VARIETY.

In 1918 the English "Mummy" pea was used in crosses with Gradus rogue and Gradus type. Seed of the Mummy variety was first obtained from O. E. White of the Brooklyn Botanical Garden (White's No. P1-3-4-2), and only this strain has been used in the crosses. In Mummy, fasciation of the main stem is characteristic, but does not appear until the plant is well developed. With the appearance of the fasciated condition the stipules become narrower and more pointed than those at the lower and middle nodes of the same plant. Plate 2, A, B, C is of a mature plant of Mummy. Although having stipules much narrower than in Gradus type, no rogue comparable to those found in Gradus have ever been observed by the writer among the several thousand



TABLE VIII.—*Gradus*×*Mummy* and *Gradus rogue*×*Mummy* in the  $F_2$  generation (aa×AA)

## TYPE×MUMMY

	AA, Aa.	aa.	Total.
Observed.....	563	179	<sup>a</sup> 742
Percentage found.....	75.88	24.12	.....
Percentage expected.....	75.00	25.00	.....
Difference (D).....	0.88	0.88	.....
Standard error (S. E.).....	1.17	.....	.....
D/S. E. ....	0.75	.....	.....

## ROGUE×MUMMY

	1178	356	1,534
Observed.....	1178	356	1,534
Percentage found.....	76.79	23.21	.....
Percentage expected.....	75.00	25.00	.....
Difference (D).....	1.79	1.79	.....
Standard error (S. E.).....	.74	.....	.....
D/S. E. ....	2.41	.....	.....

<sup>a</sup> Data from 25  $F_2$  families including 2 families (34 plants) classified as to color segregation and measured for ratio of width to length of stipule but not classified as to stipule shape, i. e., as types or intermediates.

## RELATION OF COLOR FACTORS A AND C

The  $F_1$  plants of *Gradus* type×*Mummy* were all purple-flowered. The  $F_2$  generation gave the expected recombinations in approximately the proportion 56.25 per cent purple (with factors A, B, and C), 18.75 per cent pink (with factors A and B), and 25.00 per cent white (with factors aa), the observed number in each class being in sufficient agreement with that theoretically expected when two factors are independently inherited (Table IX).

TABLE IX.—Segregation of factors for flower color in  $F_2$  generation of cross *Gradus* with *Mummy* and of *Gradus rogue* with *Mummy* (aaBBCC×AABBcc)

## GRADUS×MUMMY

	Purple ABC.	Pink ABc.	White aBc, aBC.	Total.
Observed.....	406	157	179	742
Percentage found.....	54.72	21.16	24.12	.....
Percentage expected.....	56.25	18.75	25.00	.....
Difference.....	1.53	2.41	.88	.....
$\chi^2=2.8854$ .				
P=.3405.				

## GRADUS ROGUE×MUMMY

	873	305	356	1,534
Observed.....	873	305	356	1,534
Percentage found.....	56.91	19.88	23.21	.....
Percentage expected.....	56.25	18.75	25.00	.....
Difference.....	.66	1.13	1.79	.....
$\chi^2=3.0225$ .				
P=0.2214.				

From seeds of 25  $F_1$  hybrids, 742  $F_2$  hybrids were matured in 1920. A total of 708 individuals, belonging to 23 families, were classified according to stipule shape as Graduslike types (yy) and intermediates (YY or yy), the latter class being made up of plants resembling Mummy in stipule character. The remaining families, consisting of 34 plants, were not directly classified in the same way, but the mean stipule ratio per plant was calculated from measurements of the width and length of the stipules. A record of the flower color was kept for all the 742  $F_2$  plants (Tables VIII and IX).

Of the 708 plants classified according to stipule shape, 540 were grouped as intermediates resembling Mummy and 168 as Graduslike types (Table X), indicating a single factor difference, in regard to stipule shape, between Gradus type and Mummy. The expected percentage in the two groups is 75 per cent intermediates and 25 per cent types; the observed percentages, 76.27 per cent intermediates and 23.73 per cent Graduslike types, in the  $F_2$ , are well within the limits of the standard error. The standard error as used here is calculated according to the formula of Yule (16).

TABLE X.—Segregation of stipule shape in  $F_2$  generation of Gradus  $\times$  Mummy (yy  $\times$  YY)

GRADUS $\times$ MUMMY			
	YY, Yy	yy	Total.
Observed.....	540	168	708
Percentage found.....	76.27	23.73	.....
Percentage expected.....	75.00	25.00	.....
Difference (D).....	1.27	1.27	.....
Standard error (S. E.).....	1.09	.....	.....
D/S.E.....	1.16	.....	.....

## RELATION OF Y AND y, FACTORS FOR STIPULE SHAPE

In all, 37  $F_1$  plants were grown in 1919 from the cross Gradus type with Mummy. In size and shape the stipules of the  $F_1$  plants were intermediate between Mummy and Gradus type. The upper part of a branch of a mature  $F_1$  plant is shown in Plate 3, A.

## LINKAGE RELATION OF Yy AND Aa

The relation of the factor pairs Yy and Aa was determined from a count of 708  $F_2$  plants of the cross Gradus type with Mummy (aayy  $\times$  AAYY). In Table XI are tabulated the observed numbers of the  $F_2$  recombinations. The actual ratios of the four classes AY, aY, Ay, and ay indicate that factors AY and ay are linked. The calculated zygotic series is approximately 66 AY : 9 Ay : 9 aY : 16 ay; the gametic series approximately 4 : 1 : 1 : 4; and the percentage of crossing-over for all the  $F_2$  families is  $20.41 \pm 1.28$  per cent, using Haldane's method (6) for the determining of zygotic and gametic series from observed  $F_2$  frequencies.

TABLE XI.—Linkage relation of Aa and Yy from  $F_2$  generation of Mummy  $\times$  Gradus

	aayy $\times$ AAYY.				Total.
	AY.	Ay.	aY.	ay.	
Observed.....	476	63	64	105	708
Calculated.....	467	64	64	113	.....
Difference.....	9	1	0	8	.....
$\chi^2 = 0.7554$ .....	.....	.....	.....	.....	.....

Plates 4 A, B, and 5 A, B, are of the upper parts of a Graduslike type and of an intermediate segregate, respectively, from the  $F_2$  of Gradus  $\times$  Mummy. The intermediate shown has stipules wider than the stipules of the  $F_1$  plant of the same cross shown in Plate 3, A. The difference is largely due to the fact that the stipules of the  $F_1$  plant are on a branch while those of the  $F_2$  intermediate are on the main stem. Stipules on a branch are smaller and narrower than those of the main stem in nearly all instances, irrespective of the variety or strain.

It should be pointed out here that Y and y are considered as the factors chiefly responsible for the difference for stipule shape between Mummy and Gradus. It is highly probable, from the increased variability of the  $F_2$  generation over that of the parents, that a number of modifying factors interact with Y and y to affect stipule shape.

#### RELATION OF FACTORS Aa FOR FLOWER COLOR AND Nn FOR CHARACTER OF STEM

It has been said before that stipules from the fasciated portion of the stem of Mummy are narrower than those of the apparently nonfasciated part of the same plant or from a normal plant. The  $F_1$  plants of the crosses of Mummy with Gradus were normal stemmed and had narrow, intermediate, or Mummylike stipules. No accurate counts were made of the fasciated  $F_2$  segregates, but apparently the expected 3 : 1 ratio of normal to fasciated plants occurred.

Since fasciated plants appeared, on the whole, to have narrower stipules than normal plants, it might be suggested that linkage existed between n and A, and N and a, so that the existence of a factor pair Yy would not have to be assumed. That such is not the case is shown by the results of White (14), who found factors Aa and Nn to be independently inherited. The two cases are comparable as the AA $\text{nn}$  parent of the hybrids studied by White was from the same strain as that used by the present writer.

#### STATISTICAL EVIDENCE OF LINKAGE BETWEEN FACTORS AY AND ay

In addition to the stipule measurements of the 34 plants mentioned in the preceding section, stipules of 41 other plants were measured from the  $F_2$  generation of Gradus  $\times$  Mummy. Of the 75 plants measured (Table VII), 53 were A segregates (39 purples and 14 pinks) and 23 were a segregates (whites). The mean stipule ratio of the  $F_2$  plants as a whole was found to be  $1.868 \pm 0.0126$ , for the A's  $1.905 \pm 0.0142$ , and for the a's  $1.710 \pm 0.0260$ . Considering a difference between the means of at least three times the probable error of the difference as being significant, it is found that the difference between the means of the A and a segregates is significant, since it is five times the standard error.

The same relation holds between the mean ratio of the colored and white  $F_2$  segregates, whether the A plants be considered collectively, or separately as purples (with the factors A and B) and as pinks (with the factors A and b), the mean of the purples being  $1.909 \pm 0.0175$  and of the pinks  $1.864 \pm 0.0142$  as compared with  $1.905 \pm 0.0142$  for the A segregate as a whole (Table VII).

No essential difference exists between the means of Gradus and the white-flowered (aa)  $F_2$  segregates from the cross Mummy with Gradus. However, the hybrid whites show a greater variability than do the pure whites, the coefficient of variation of the former being  $10.58 \pm 1.0870$  compared with  $5.78 \pm 0.2381$  for the latter.

The increased variation of the  $F_2$  aa plants is probably due for the most part to crossing-over of the factors Yy for stipule shape and partly to the recombination of modifying factors affecting the same character.

The mean ratio of the A segregates,  $1.905 \pm 0.0142$ , is significantly less than that of the Mummy parent,  $2.088 \pm 0.0050$ , and the variability of the former is greater than that of the pure AA plants. The increased variability of the colored-flowering segregates, besides being accounted for largely by crossing-over, could, in addition, be due to a dissimilarity in stipule shape of AA and Aa plants. The heterozygous plants being in excess would tend to lower the mean of the population. Actually the  $F_1$  plants did appear to have stipules intermediate in shape between those of the two parents, but no measurements are available for comparison.

It is realized that the number of  $F_2$  individuals measured is small but, taken alone, the statistical results would offer fairly good evidence of linkage between the factor or factors for stipule shape and the A factor for flower color. In connection with the  $F_2$  classification based on stipule shape and flower color, the fact of linkage between the Aa and Yy factors is well established.

#### GRADUS ROGUE $\times$ MUMMY

The Gradus rogues used in the series of crosses with Mummy were all descended from a rogue plant, S15, selected in 1916 from a commercial planting of Gradus. Progeny of the same rogue, S15, when crossed with Gradus type behaved as typical rogues in inheritance (cultures 9.1270-9.1275, 9.1320, and 9.1321, Table II).

The  $F_1$  plants (Table XII) of Gradus rogue  $\times$  Mummy, and reciprocal had, with one exception, rogue stipules, and in this respect appeared similar to the pure rogues. As volunteer crossing occurred in the  $F_1$  of Gradus rogue  $\times$  Gradus, it might be expected to take place among the  $F_1$  hybrids of rogue and Mummy. Since the  $F_1$  plants in the latter cross exhibited most of the dominant Mendelian factors present in any of the strains growing in the experimental gardens in 1919, it would be difficult to detect volunteer hybrids in the  $F_2$  generation ( $F_1$  out-crosses). However, as the  $F_1$  hybrids were very late in flowering, cross-pollination by insects, if not entirely absent was cut down to a minimum.

TABLE XII.—Pedigrees of  $F_1$  cultures of Gradus rogue  $\times$  Mummy (P16) and reciprocal

Culture.	Pedigree.	Mature plants.
9.720	(S15-2-5r $\times$ P16-10)—1 to 6.....	Rogues.
9.721	(S15-2-12r $\times$ P16-10)—1 to 9.....	Do.
9.722	(S15-2-3r $\times$ P16-10)—1 to 7.....	Do.
9.723	(S15-2-19r $\times$ P16-10)—1 to 6.....	Do.
9.725	(S15-1-6r $\times$ P16-10)—1 to 3.....	Do.
9.726	(S15-1-9r $\times$ P16-10)—1 to 8.....	Do.
9.727	(S15-3-1r $\times$ P16-10)—1 to 8.....	All rogues but No. 6, which resembled $F_1$ of P 16 $\times$ type.
9.728	(S15-3-4r $\times$ P16-10)—1 to 3.....	Rogues.
9.729	(S15-3-5r $\times$ P16-10)—1 to 8.....	Do.
9.730	(P16-12 $\times$ S15-4-5r)—1 to 11.....	Do.
Total.....		69

As some uncontrolled pollinations may have occurred, there is possibly an experimental error in the results which should be kept in mind when interpreting the  $F_2$  data. On this account the crosses are being repeated, and it is planned to grow the  $F_1$  generation under glass for protection. It is believed, however, that the data here presented are of sufficient interest to warrant publication at this time and that the errors from out-crossing will prove minimal.

#### RELATION OF THE A AND A FACTORS FOR FLOWER COLOR

The  $F_1$  plants of the crosses between rogue and Mummy had colored (purple) flowers. In Table VIII is shown the percentage of colored-flowered (AA + Aa), segregates to white-flowered (aa), segregates in the  $F_2$  generation of rogue  $\times$  Mummy, and reciprocal. The observed percentages, 76.79 per cent AA and Aa plants and 23.21 per cent aa segregates conform to expectation.

#### RELATION OF A AND C, FACTORS FOR FLOWER COLOR

Gradus rogue  $\times$  Mummy in the  $F_2$  generation gave approximately the expected 56.25 per cent purples (with factors A, B, and C); 18.75 per cent pinks (with factors A and B); and 25.00 per cent whites (with factors aa), the observed percentages being 56.91 per cent, 19.88 per cent and 23.21 per cent, respectively, the deviations being well within the limits of error due to random sampling (Table IX).

Although the dovetailing of observation and theory is good for the three classes of color segregates as a whole, there is in the  $F_2$  in both the case of Mummy  $\times$  Gradus and Mummy  $\times$  Gradus rogue, a deficiency of white segregates. The deficiency of aa plants can be explained by differences in disease resistance as well as by variations in time of maturing between colored and noncolored  $F_2$  segregates. On the whole, plants with colored flowers are more resistant to disease than are the white flowered forms, so that proportionately more of the latter would be lost before they reached maturity. For unavoidable reasons the  $F_2$  data were collected late in the season after a number of the plants had matured. Plants whose stipules were too dried to measure were not classified as to flower color but their occurrence was noted. Tschermak (12), Hoshino (7), and others have noted a correlation between the presence of the A factor and the habit of late flowering. In all probability the same correlation existed in these cultures. On this account, relatively more of the white segregates than of the colored ones were too mature to be used in this study at the time the stipule measurements were made.

#### RELATION OF N AND N FACTORS FOR NORMAL AND FASCIATED STEM, RESPECTIVELY

The 69  $F_1$  plants of Gradus rogue  $\times$  Mummy had normal stems. No  $F_2$  counts were made of the normal and fasciated plants, but apparently there occurred the expected Mendelian segregation.

#### RELATION OF $Xx'$ AND $Yy$ FACTORS FOR STIPULE SHAPE

In 1919, 69  $F_1$  hybrids (Table XII) were grown from the seed of 13 pollinations made the preceding year. The young plants resembled Mummy in regard to stipule shape and were uniform in appearance.

With the exception of one plant (9.727-6), the hybrids were all classified as rogues at maturity. Plate 3, B, is of the upper part of a branch of a mature  $F_1$  plant from Mummy  $\times$  Gradus rogue.

The exceptional plant (9.727-6) resembled the  $F_1$  of Mummy  $\times$  Gradus, although 7 other plants from the same cross had rogue stipules. The atypical  $F_1$  hybrid (9.727-6) produced 61  $F_2$  plants in 1920 (Culture No. 0.1084). At the time the notes were taken 14 individuals were too dried to classify. Of the 47 remaining plants, 23 were grouped as intermediates, with stipules intermediate in shape between those of Mummy and those of Gradus, and 24 were classed as Graduslike types. Apparently there were no rogues among the  $F_2$  offspring.

Besides the  $F_2$  family (0.1084) derived from the atypical  $F_1$  plant, five other  $F_2$  cultures were classified by inspection as rogues and non-rogues, the latter being subdivided into broads and intermediates (Tables XIII and XIV). The number of individuals in each of the three categories is shown in Table XIV. The ratio of rogues to non-rogues indicates a single factor difference between the two parents as being primarily responsible for the difference in stipule shape. The observed ratio of 77.12 per cent rogues to 22.88 per cent nonrogues agrees with that theoretically expected being within the error due to the limitations of random sampling.

On the assumption of a single factor pair,  $X$  and  $x'$ , primarily responsible for a difference in mean stipule ratio of 2.05 in Mummy and as compared with 2.35 in Gradus rogue, one would expect the  $F_2$  to segregate only into rogues and Mummylike plants. Actually three classes based on stipule shape were distinguished, namely, rogues, intermediates (Mummylike), and broads (Table XIV), the latter having stipules comparable to those of Gradus.

TABLE XIII.—Classification into rogues and nonrogues of five  $F_2$  families from the cross *Gradus rogue*  $\times$  *Mummy*

Parent.	Culture No.	Rogues.	Nonrogues.		Unclassified.	Total.
			Intermediate.	Broad.		
1919:	1920:					
9.726-5.....	0.1076.....	79	19	3	8	109
9.726-6.....	0.1077.....	68	15	2	1	86
9.726-7.....	0.1078.....	8	4	2	2	16
9.726-8.....	0.1079.....	11	4	0	4	19
9.727-8.....	0.1085.....	16	4	1	6	27
		182	46	8	21	257

TABLE XIV.—Relation of  $Xx'$  and  $Yy$  in  $F_2$  generation of cross Mummy  $\times$  Gradus rogue ( $x'x'YY \times XXyy$ ), data from Table XIII.

	XY or Xy rogues.	x'Y or x'y nonrogues.		Total.
		Intermediate.	Broads.	
Observed, 12:3:1 ratio .....	182	46	8	236
Percentage found.....	77.12	19.49	3.39	.....
Percentage expected.....	75.00	18.75	6.25	.....
Difference (D).....	2.12	.74	2.86	.....
$\chi^2=2.8587$				
$P=.2231$				
Observed, 3:1 ratio.....	182	54	.....	236
Percentage found.....	77.12	22.88	.....	.....
Percentage expected.....	75.00	25.00	.....	.....
Difference (D).....	2.12	2.12	.....	.....
Standard error (S. E.).....	1.89	.....	.....	.....
D/S. E. ....	1.12	.....	.....	.....

The presence of the broads in the  $F_2$  generation of the cross Gradus rogue with Mummy is accounted for by assuming the presence of two factors  $y$  and  $x$  for stipule width in Gradus, factor  $y$  being allelomorphous to  $Y$  in Mummy and factor  $x$  allelomorphous to  $x'$  found in Mummy. Ordinarily neither  $x$  nor  $x'$  has any visible effect on stipule shape in either variety. Occasionally the factor  $x$  in Gradus mutates to  $X$ , which has for its somatic expression a mean stipule ratio of about 2.35. Factor  $X$  interacts with its allelomorph  $x$  as found in Gradus and is dominant to  $x'$  of Mummy. It also masks either member of the factor pair  $Yy$ . Any  $F_2$  segregate that is heterozygous or homozygous for the factor  $X$  has rogue stipules, irrespective of whether  $Y$  or  $y$  is present. An individual in which  $X$  is absent has intermediate or broad stipules, depending on the presence or absence, respectively, of the factor  $Y$ . In the  $F_2$  generation the plants segregate into approximately 12 rogues; 3 intermediates; 1 broad, according to the following scheme:

$P_1$ .....	Gradus rogue, $XXyy \times$ Mummy, $XXx'x'$			
$F_1$ .....	Rogue, $Xx'Yy$			
$F_2$ .....	Rogues: Intermediates: Broads:			
	$Xy$	$Xy$	$x'Y$	$x'y$
Percentage.....	56.25	18.75	18.75	6.25
Ratio.....	12	:	3	:
	Rogues (X):		Non-rogues (X'):	
Percentage.....	75		25	
Ratio.....	3		1	
	Rogues and Intermediates:		Broads:	
Percentage.....	93.75		6.25	
Ratio.....	15		1	

Returning again to the results tabulated in Table XIV, the observed number in any one category of plants is expressed in percentages of the total number of plants classified. For the rogues, intermediates, and types the percentages are 77.12, 19.49, and 3.39, respectively, compared with the theoretically expected 75.00, 18.75, and 6.25 for the respective classes, the deviations of the observed percentages from the calculated are not statistically significant ( $P$  having a value of 0.2331). The greatest deviation is caused by the exceptionally low number of board-stipuled plants found. The deficiency in the broad class, however,

may be partially explained by assuming a differential mortality rate among the  $F_2$  segregates as explained in the paragraph dealing with the relation of the Aa and Cc factors in the  $F_2$  generation of Gradus rogue  $\times$  Mummy.

The occurrence of the early flowering segregates offers a partial explanation of the deviation in the number of broad-stipuled plants observed from that theoretically expected, as there is in all likelihood a correlation in Gradus and Mummy between the shape of the stipules and time of maturing. As has been shown, the Graduslike character of the broad-stipuled  $F_2$  plants was inherited through the rogue parent, which also carried the factors for early flowering. The results with Gradus  $\times$  Mummy show that linkage exists in Gradus between the factors  $y$  for stipule shape and  $a$  for flower color. Hoshino (7) has shown the factor  $a$  to be linked with a factor for early flowering. Necessarily linkage exists in some degree between the factor  $y$  for stipule shape and the factor for early flowering. One is, therefore, justified in assuming that many of the white, broad-stipuled segregates would be early in maturing and the correlation would account for a part of the deviation of the observed number of broad  $F_2$  types from the number theoretically expected.

#### STATISTICAL STUDY OF THE $F_2$ GENERATION OF THE CROSSES GRADUS ROGUE AND MUMMY

In 1920 were grown 51  $F_2$  families of the cross Gradus rogue with Mummy. The average ratio of stipule width to length in the mature  $F_2$  plants was calculated in the same manner as for the  $P_1$  generation. The  $F_2$  segregates were also classified according to flower color but not described as broads, intermediates, or rogues.

#### RELATION OF COLOR FACTORS A AND a TO STIPULATE SHAPE IN TWO ATYPICAL FAMILIES

With the exception of the plant already mentioned,<sup>5</sup> all of the  $F_1$  hybrids were rogues and appeared similar in regard to stipule shape. Although somatically alike the  $F_2$  frequency distributions of the progeny of two apparently normal  $F_1$  rogues are different from those of the remaining 49  $F_2$  families in both their range of variation and mean stipule ratio (Table XV). The means of the two families are  $1.999 \pm 0.9106$  and  $1.905 \pm 0.0126$  and for the two together the mean is  $1.998 \pm 0.0082$  as compared with  $2.333 \pm 0.0050$  for the other 49  $F_2$  cultures (Table XVI).

Because of their similarity both in frequency distribution and in the value of the mean stipule ratios, the two anomalous cultures have been lumped together for a statistical study of the color segregates. The means of the three color classes are: For the whites,  $1.899 \pm 0.0159$ ; for the pinks,  $2.046 \pm 0.0167$ ; for the purples,  $2.038 \pm 0.0095$ ; and for colored plants as a whole,  $2.027 \pm 0.0085$ . The difference in means of the AA and aa as compared with aa plants is 6.7 times the probable error. The frequency distribution of the a plants resembles more that of Gradus than it does that of Mummy or Gradus rogue. The frequency distribution of the A population resembles that of Mummy rather than that of either Gradus or Gradus rogue. The difference in the mean stipule ratios of the two color groups, as well as the difference in their ranges

<sup>5</sup> The two exceptional  $F_2$  families were cultures numbered 0.1059 and 0.1093 (Table XV), the progeny of  $F_1$  plants 9.722-2 and 9.722-5, respectively (Table XII).



TABLE XV.—*Gradius roque* × *Mummy*, and reciprocal. Frequency distributions of ratio of  $\frac{\text{length of stipeule}}{\text{width of stipeule}}$  of  $F_2$  families

Culture No.	1 55	1 65	1 75	1 85	1 95	2 05	2 15	2 25	2 35	2 45	2 55	2 65	2 75	2 85	3 05	Total.	M.	Coefficient variation.
o.1045																35	2.325 ± 0.0329	12.43 ± 0.9848
o.1046		1	0	1	4	4	3	1	5	7	2	3	2	2		23	2.245 ± 0.0374	11.85 ± 1.1949
o.1047		1	1	1	0	0	1	4	3	6	3	1				11	2.359 ± 0.0390	8.14 ± 1.6217
o.1048				1	1	1	0	0	4	3	3	1	0	0	1	11	2.466 ± 0.0331	10.75 ± 0.7151
o.1049			1	1	1	3	4	5	7	7	11	12	3	3	1	59	2.396 ± 0.0350	11.48 ± 0.850
o.1050			1	1	0	2	5	4	4	2	7	6	8	3	0	44	2.340 ± 0.0364	10.59 ± 0.8275
o.1051			1	1	1	4	1	5	9	9	2	3	2	1		50	2.283 ± 0.0245	11.56 ± 0.7674
o.1052			1	5	2	6	6	5	10	8	4	3	2	1		43	2.358 ± 0.0407	10.98 ± 1.4190
o.1053			1	1	2	2	1	0	1	4	1	2	1			14	2.030 ± 0.0568	10.95 ± 1.9973
o.1054			1	1	2	1	0	1	0	1						7	2.410 ± 0.0209	9.95 ± 1.8113
o.1055			1	3	4	3	3	5	6	5	11	9	2	4	3	60	2.320 ± 0.0295	10.14 ± 0.9072
o.1056			1	1	2	3	3	4	5	4	1	1	2			29	2.312 ± 0.0215	11.72 ± 0.6580
o.1057			1	5	3	7	9	0	6	17	6	8	1	2		72	2.215 ± 0.0312	11.06 ± 1.0033
o.1058			1	1	1	9	5	1	2	5	1	0	2			28	2.340 ± 0.0397	7.94 ± 1.2142
o.1059			1	1	1	2	0	2	2	2	1	1				10	1.999 ± 0.0106	6.65 ± 0.3781
o.1060			1	3	9	23	1	0	2	1	1					71	2.350 ± 0.0494	6.97 ± 1.5001
o.1061			2	0	3	3	1	0	1	4	6	1				5	2.000 ± 0.0358	8.40 ± 1.2758
o.1062			1	2	1	1	1	1	3	4	1			2		25	2.370 ± 0.0362	11.35 ± 1.0956
o.1063			1	1	0	1	1	1	1	2	4	1				12	2.160 ± 0.0322	12.41 ± 1.7348
o.1064			1	1	1	0	2	4	4							12	2.268 ± 0.0302	7.02 ± 0.9084
o.1065			1	5	5	1	1	7	7	7	4	2	0	3		52	2.255 ± 0.0265	12.59 ± 0.8459
o.1066		1	0	1	3	1	1	3	4	3	3	3	1			24	2.312 ± 0.0370	11.03 ± 1.1492
o.1067		1	1	1	3	1	1	6	7	7	9	8	5			57	2.357 ± 0.0256	12.17 ± 0.7798
o.1068		1	1	2	3	2	4	1	2	4	10	7	1	2		40	2.357 ± 0.0322	12.81 ± 0.9817
o.1069		2	3	4	6	2			1							15	2.215 ± 0.0313	12.59 ± 1.0165
o.1070				1	1	0	2	2	1	1						8	2.175 ± 0.0416	8.41 ± 1.4282
o.1071				2	0	2	3	1	4	0	4	1	2			19	2.328 ± 0.0414	11.51 ± 1.2760
o.1072					0	2	1	2	1	2	0					10	2.390 ± 0.0314	6.65 ± 0.9908
o.1073					1	0	0	0	2	4	0	0	1			5	2.310 ± 0.0552	7.92 ± 0.6998
o.1074			1	2	3	6	2	6	2	12	4	2	1	2		41	2.399 ± 0.0248	10.22 ± 0.7691
o.1075						4	0	1	1	4	2	0				14	2.372 ± 0.0386	9.02 ± 1.1593
o.1076				1	0	0	2	1	4	2	0	1	0	1		13	2.397 ± 0.0245	11.72 ± 1.5715
o.1077								2	2	1	3	2	1	0	2	14	2.543 ± 0.0368	8.69 ± 1.1106
o.1078				1	3	4	1	2	6	11	5	8	2	2		49	2.434 ± 0.0251	9.90 ± 0.7350
o.1079				1	0	2	0	1	4	8	7	7	3	1	0	40	2.470 ± 0.0254	9.63 ± 0.7359
o.1080				1	0	2	0	1	3	1	1	1	2	1		9	2.317 ± 0.0607	11.65 ± 1.9772
o.1081				1	0	2	3	1	3	1	1	3	2	1		19	2.350 ± 0.0479	13.19 ± 1.4682
o.1082				1	0	2	3	0	4	4	1	1	1	1		19	2.472 ± 0.0385	10.07 ± 1.1050
o.1083				2	6	3	5	6	7	11	10	3	3	2	1	60	2.350 ± 0.0245	11.91 ± 1.4496
o.1084				1	5	1	1	1	6	5	6	5	2	0		34	2.303 ± 0.0304	11.41 ± 0.9453
o.1085				1	0	2	1	1	5	1	1	1	1			15	2.250 ± 0.0406	10.35 ± 1.3893
o.1086				1	0	1	1	0	1	1						5	2.170 ± 0.0642	9.81 ± 1.1125

o.1093 <sup>a</sup> .....	2	4	5	18	21	6	0	0	2	1	4	1	...	...	...	...	59	1 905± .0159	7.72± .4822
o.1094.....	2	2	11	15	5	3	0	0	4	2	4	1	...	...	...	...	53	2.094± .0224	11.55± .7667
o.1095.....	1	0	3	1	6	1	13	0	5	18	5	1	3	1	1	...	71	2.366± .0173	9.30± .5338
o.1096.....	...	...	...	...	2	0	0	0	0	2	...	...	...	...	...	...	4	2.250± .0951	12.53± 3.0347
o.1097.....	...	2	7	6	8	6	14	0	10	16	10	8	9	8	7	3	119	2.385± .0181	12.88± .5725
o.1098.....	...	1	1	2	1	0	1	1	0	0	0	1	1	1	1	...	6	2.117± .0738	12.65± 2.5023
o.1099.....	...	...	...	...	1	1	1	1	4	2	2	0	3	0	1	...	14	2.471± .0326	7.32± 1.6792
o.1100.....	...	1	0	0	1	0	0	0	0	1	0	1	0	1	1	...	4	2.475± .0904	10.82± 2.6185
o.1101.....	...	1	0	0	1	2	0	0	2	0	1	0	0	0	1	...	8	2.275± .0741	13.67± 2.3478
Total.....	11	27	56	140	107	122	1.6	1.6	187	231	169	131	58	39	12	4	1,534	2.307± .0048	12.48± .1542

<sup>a</sup> Atypical cultures o.1099 and o.1093.

of variation, indicates linkage in the two parents between the factors Yy for stipule width and the Aa factors for flower color. Recombinations in the  $F_2$  of cross-over and noncross-over gametes would account for differences in the range of variation and in the means between the  $F_2$ , A and a segregates and the A and a parents.

However, as the combined frequency distributions of the two crosses of Mummy with rogue cultures under discussion (0.1059 and 0.1093) show considerable variation from the  $F_2$  frequency distribution of Gradus as type  $\times$  Mummy, especially in the position of the respective modes and means, it is unlikely that the factor allelomorphic to  $x'$  in the  $F_1$  hybrid is the  $x$  of Gradus type; it is apparently a mutation of  $X$  to some other factor for stipule shape, the mutation being analogous to the genetic change occurring when intergrading intermediates are produced from Early Giant types (1). In this case the intermediates are of the sort producing few or no rogues among their progeny.

Although the ranges of variation of the two atypical  $F_2$  progenies (0.1059 and 0.1093) overlaps the range of Gradus rogue, no rogues were present in the cultures judging from the character of the  $F_3$  generation. Six  $F_2$  plants of culture 0.1093 from classes 2.05, 2.15, and 2.45 of the variation curve gave only broad and intermediate  $F_3$  segregates. It would appear that the rogue factors which entered the  $F_1$  zygote failed to function as rogue factors at gametogenesis, having mutated to something different from  $X$ .

RELATION OF COLOR FACTORS A AND C TO STIPULE SHAPE IN THE TYPICAL  $F_2$   
FAMILIES OF THE CROSSES OF MUMMY WITH GRADUS ROGUE

The stipules of 1,404 plants, progeny of the remaining  $F_2$  families (omitting cultures 0.1059 and 0.1093), were measured. The plants represented 49 families, of which the frequency distributions and the derived statistical constants are given in Table XV.

Of the 1,404 individuals, 356 were white (of constitution aa); 305 pink (with A and B factors); and 873 were purple (with A, B, and C factors). The mean stipule ratio of the white segregates was found to be  $2.258 \pm 0.0110$  as compared with  $2.336 \pm 0.0110$  for the pinks and  $2.325 \pm 0.0063$  for the purples (Table VII).

As no essential difference exists between the means of the pink and purple  $F_2$  plants, and as they each contain the A factor, the two color populations may be combined for comparison with the aa segregates. The mean of the AA and Aa plants is  $2.335 \pm 0.0056$  as contrasted with  $2.258 \pm 0.0110$  for the aa segregates, a difference in the means of over 6 times the probable error of the difference.

For a comparison inter se of the frequency distributions of the AA and Aa, and aa segregates and of the  $F_2$  as a whole the number of variates in each class was expressed in percentages of the total number of variates in that particular category of plants. Figure 3 shows the curves obtained by plotting the percentage of variates in each class for the AA and Aa, and aa segregates separately, and for all the  $F_2$  plants irrespective of the color factors present. The graphs for Gradus, Gradus rogue, and Mummy are shown in figure 2, for comparison with the graphs of the  $F_2$  generation.

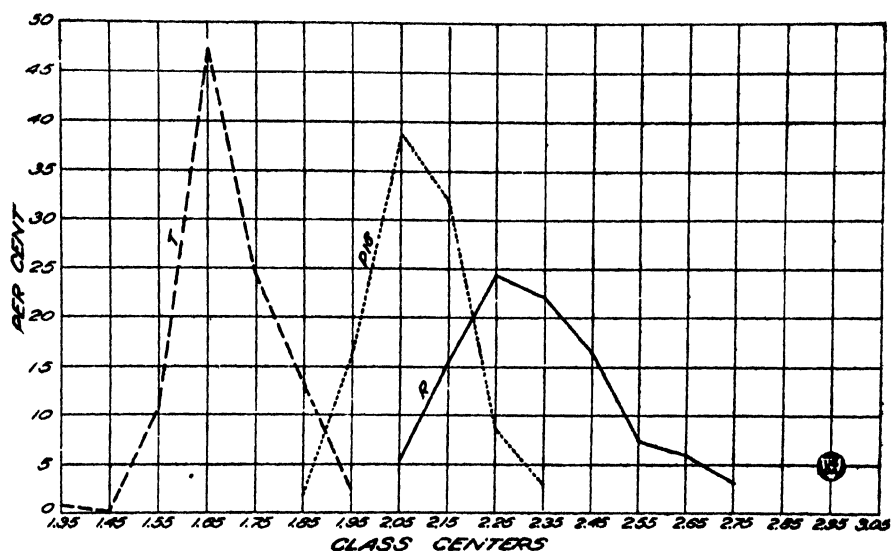


FIG. 2.—Graphs obtained by plotting the frequency distributions, expressed in percentages, of ratio of length of stipule to width of stipule for Gradus (T), Mummy (M), and Gradus rogue (R), based on data in Table VIII.

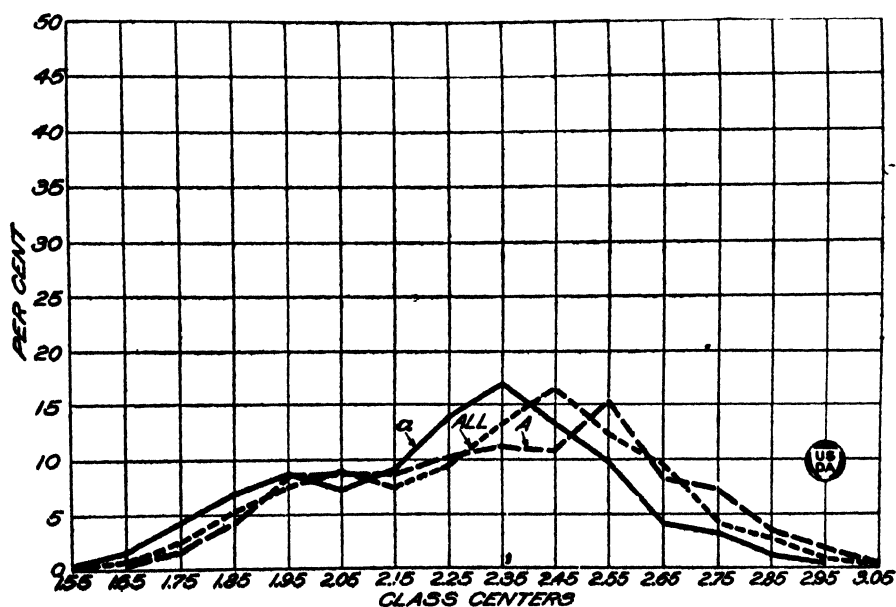


FIG. 3.—Graphs obtained by plotting the frequency distributions, expressed in percentages, of ratio of length of stipule to width of stipule for all the  $F_2$  variates of Mummy  $\times$  Gradus rogue and for the AA and Aa segregates (A) and aa segregates (a) taken separately, based on data in Table VIII.

The majority of variates in a population of Gradus types have a mean stipule ratio between 1.50 and 1.79 and none have been observed with a stipule ratio above 1.99. For Gradus rogue no plants have been found with a stipule ratio of less than 2.00 and by far the greatest number occur between the limits 2.10 and 2.49. The mean stipule range of Mummy extends over portions of the ranges of both Gradus and Gradus rogue, but the majority of individuals occur in classes 1.95 to 2.15, inclusive. Consequently, if linkage existed between the factor for rogue stipules and the a factor for white flowers, one would expect proportionately more of the aa variates to lie close to the mean of the rogue parent, 2.339, and a greater concentration of the variates with the A factor around the mean of Mummy, 2.088, than would be found close to the mean of Gradus rogue. The modes of the frequency distributions of both the colored (AA and Aa) and white (aa) segregates are actually at 2.45, or very near the mean of the rogue parent, however. It is evident, therefore, that no linkage exists between the factor for rogue stipules and the a factor.

The difference in means of the two populations is caused in a large measure by the linkage of the AY and ay factors, so that among the non-rogue segregates the AA and Aa variates tend to have a stipule ratio approaching that of Mummy and the mean of the aa segregates approaches that of the Gradus parent. The effect of linkage is to lower the mean of the aa plants and to increase the mean of the AA and Aa segregates. For this reason more aa segregates than AA and Aa segregates are found in classes 1.55 to 1.95, inclusive. Linkage of the two factors also accounts for the tendency to bimodality that both curves exhibit. (Fig. 3.)

GRADUS ROGUE  $\times$  MUMMY, AND RECIPROCAL IN THE  $F_2$  GENERATION;  
CLASSIFICATION OF  $F_2$  FAMILIES

On the hypothesis that Gradus rogues are produced from Gradus by mutation of a single factor, x to X, which is allelomorphic to x' in Mummy, the expectation in regard to the  $F_2$  rogues of Gradus rogue  $\times$  Mummy is that approximately 66 per cent will show segregation in the  $F_2$  generation into rogues and nonrogues, while the remaining 33 per cent should breed true. The  $F_2$  broads and intermediates (nonrogues) should not contain rogues among their progeny except as the product of primary mutation.

No record was kept of the  $F_2$  plants saved for seed as to their general appearance, i. e., whether they were rogues, intermediates, or broads. Instead, stipule measurements were made and the stipule ratio was used for a criterion as to which of the three categories, rogue, intermediate, or broad, that the plant belonged. Altogether 184 plants were chosen from a number of  $F_2$  frequency classes (Tables XVI to XVIII). As expected, the parent plants of families containing no rogues came for the most part from classes 1.65 to 2.25. The parent plants of progenies breeding true to the rogue character or segregating into rogues and nonrogues were largely from classes 2.35 to 2.95. This and a classification of segregating  $F_2$  families of Gradus rogue  $\times$  Mummy and reciprocal are shown in Table XVI.

TABLE XVI.—Families in which no rogues appeared. Generic constitution of  $F_2$  parents was  $x'x'YY$ ,  $x'x'Yy$ , or  $x'x'yy$ 

Culture No.	Stipule ratio of $F_2$ parent.	Broads.	Broads and intermediates.	Intermediates.	Total.
0.279		9			9
0.281	1.65	29			29
0.283			31		31
0.285				13	13
0.289			16		16
0.291		27			27
0.293			25		25
0.294			16		16
0.296			44		44
0.297			35		35
0.298	1.75		21		21
0.299		18			18
0.300			24		24
0.301			61		61
0.302			49		49
0.303			33		33
0.304				38	38
0.305				37	37
0.306			35		35
0.308			12		12
0.309			37		37
0.310		13			13
0.311		46			46
0.314		21			21
0.315			23		23
0.316			73		73
0.317	1.85		44		44
0.318			21		21
0.319			25		25
0.321			38		38
0.322			91		91
0.323			62		62
0.324			70		70
0.325			20		20
0.326			38		38
0.327			74		74
0.328			11		11
0.330			47		47
0.331			20		20
0.332			22		22
0.340			71		71
0.341	2.05	58			58
0.344			30		30
0.345			47		47
0.347			25		25
0.348			23		23
0.428			75		75
0.429			31		31
0.432	2.25		42		42
0.433			110		110
0.454	2.35		99		99
0.466	2.45		42		42

TABLE XVII.—*Families segregating into rogues and nonrogues. Genetic constitution of  $F_2$  parents was  $Xx'YY$ ,  $Xx'Yy$ , or  $Xx'yy$* 

Culture No.	Stipule ratio of $F_2$ parent.	Rogues.	Nonrogues.	Total.
0.307	1.85	31	11	42
0.329		18	8	26
0.333		?	?	70
0.418		?	?	38
0.420		18	2	20
0.421	2.25	16	3	19
0.422		27	3	30
0.425		31	2	33
0.426		69	4	73
0.431		34	4	38
0.436		18	2	20
0.440		24	3	27
0.441		21	2	23
0.443		19	3	22
0.446		31	6	37
0.450		33	2	35
0.451	2.35	34	3	37
0.453		32	2	34
0.457		57	3	60
0.459		36	2	38
0.460		117	13	130
0.461		64	5	69
0.462		33	4	37
0.465		88	6	94
0.466		25	4	29
0.470		23	5	28
0.471		25	3	28
0.472		14	9	23
0.473		29	3	32
0.474		12	1	13
0.477		13	4	17
0.482	2.45	15	8	23
0.484		29	4	33
0.486		22	6	28
0.489		28	7	35
0.491		24	7	31
0.492		37	9	46
0.493		14	2	16
0.497		19	4	23
0.500		18	2	20
0.502		6	5	11
0.503		16	5	21
0.2419		66	9	75
0.2420		36	5	41
0.2423		28	14	42
0.2424		58	3	61
0.2427		46	3	49
0.2428		25	6	31
0.2429	2.55	26	3	29
0.2430		11	4	15
0.2434		51	2	53
0.2439		?	?	36
0.2441		?	?	11
0.2442		?	?	51
0.2443		6	3	9
0.2444		6	1	7
0.2447		25	2	27
0.2448		18	6	24
0.2449		57	3	60

TABLE XVII.—*Families segregating into rogues and nonrogues. Generic constitution of  $F_2$  parents was  $Xx'YY$ ,  $Xx'Yy$ ,  $Xx'yy$ —Continued*

Culture No.	Stipule ratio of $F_2$ parent.	Rogues.	Nonrogues.	Total.
0.2451 .....		49	12	61
0.2452 .....		29	2	31
0.2458 .....		35	2	37
0.2462 .....	2.65	10	2	12
0.2463 .....		15	3	18
0.2464 .....		20	1	21
0.2466 .....		6	2	8
0.2467 .....		9	2	11
0.2468 .....		19	4	23
0.2473 .....		5	4	9
0.2475 .....		34	3	37
0.2476 .....	2.75	31	5	36
0.2477 .....		40	8	48
0.2484 .....	2.85	19	7	26
0.2489 .....		18	1	19
0.2490 .....	2.95	39	5	44
0.2491 .....		25	2	27
Total .....		2,097	308	.....
0.423 <sup>a</sup> .....		7	57	64
0.424 <sup>a</sup> .....	2.25	4	19	23
0.430 <sup>a</sup> .....		7	23	30
0.439 <sup>a</sup> .....	2.35	4	33	37
0.455 <sup>a</sup> .....		3	67	70
0.469 <sup>a</sup> .....	2.45	4	54	58
0.574 <sup>a</sup> .....		6	8	14
0.2445 <sup>a</sup> .....	2.65	13	19	32
0.2470 <sup>a</sup> .....		12	17	29
0.2482 <sup>a</sup> .....	2.75	10	14	24
Total .....		70	311	.....
Grand total .....		2,167	619	.....

<sup>a</sup> Cultures containing an excess of nonrogues over rogues. The nonrogues were comparable to the intergrading intermediates described by Bateson and Pellew (1).

Of the 184  $F_2$  plants, 52 had progenies containing (1) only broads, (2) both intermediates and broads, or (3) intermediates only; i. e., they were recessive for the X factor but may have contained the Y or y factor. There were 86 progenies which segregated into rogues and nonrogues; while the progenies of 46 plants were all rogues (Tables XVI to XVIII). The observed number of  $XX:Xx'$   $F_2$  segregates is very close to that theoretically expected (Table XIX).

Inspection showed segregating families to be of two kinds: First, those with an excess of rogues over nonrogues, and second, those with an excess of nonrogues over rogues. In the latter case the nonrogues were often comparable in appearance to the intergrading intermediates described by Bateson and Pellew (2). Except in cultures containing a very few plants, one would not expect such an excess of recessives as was found in these families.



TABLE XVIII.—Families in which only rogues appeared. Genetic constitution of  $F_2$  parents was  $XXYY$ ,  $XXYy$ , or  $XXyy$ 

Culture No.	Stipule ratio of $F_2$ parent.	Rogues.	Culture No.	Stipule ratio of $F_2$ parent.	Rogues.
0.419.....		55	0.2425.....		42
0.427.....	2.25	49	0.2426.....	2.55	38
0.434.....		50	0.2435.....		32
0.442.....		25	0.2436.....		61
0.444.....	2.35	23	0.2437.....		44
0.445.....		52	0.2440.....		41
0.456.....		30	0.2418.....		34
0.463.....		18	0.2446.....		36
0.476.....		16	0.2450.....		16
0.478.....		29	0.2454.....		27
0.480.....		15	0.2455.....		20
0.481.....		21	0.2456.....		33
0.483.....		18	0.2457.....	2.65	28
0.485.....		26	0.2459.....		31
0.487.....	2.45	24	0.2460.....		19
0.488.....		17	0.2465.....		51
0.495.....		20	0.2469.....		17
0.496.....		13	0.2471.....		23
0.498.....		42	0.2472.....		18
0.499.....		39	0.2479.....	2.75	34
0.502.....		14	0.2483.....		44
0.2421.....		34	0.2486.....	2.85	15
0.2422.....		35	0.2487.....		17

TABLE XIX.—Ratio of  $XX : Xx'$  plants among 132  $F_2$  segregates of *Gradus rogue* × *Mummy*, as determined from analysis of the  $F_3$  generation, based on data in Table XVII. The expectation is two segregating and one nonsegregating  $F_3$  families

	Nonsegregating. <sup>a</sup> $XX$ . <sup>b</sup>	Segregating. <sup>a</sup> $Xx'$ . <sup>b</sup>	Total.
Observed.....	46	86	132
Calculated.....	44	88	132
Difference (D).....	2	2	
Standard error (S. E.).....	3.5	3.5	
D/S. E. ....	.5	.5	

<sup>a</sup> Character of  $F_3$  family.<sup>b</sup> Constitution of  $F_3$  parent.

The total number of rogues (2,097) in the cultures containing few nonrogues is obviously far in excess of the total number of nonrogues (308) that would be expected if normal Mendelian segregation occurred in the  $F_2$  plants heterozygous for factors  $X$  and  $x'$  (Tables XVI, XVII, XVIII). In the families producing many nonrogues and few rogues the situation is reversed, the cultures containing in all 70 rogues and 311 nonrogues, plainly not in accord with the theoretical, monohybrid 3 : 1 ratio shown in the same tables.

Disregarding the two types of  $F_3$  families and considering all the segregating families as a whole, the observed numbers are: 2,167 rogues ( $XX$  and  $Xx'$ ) and 619 nonrogues ( $x'x'$ ). The numbers actually found in the two groups show an excess of 78 individuals in the rogue class, a deviation of nearly 4 times the probable error of the difference between the observed and calculated number of variates (Table XX).

TABLE XX.—Ratio of rogue ( $XX$  and  $Xx'$ ) segregates to nonrogue ( $x'x'$ ) segregates in all  $F_3$  cultures segregating into rogues and nonrogues, based on data in Table XVII

	$XX$ and $Xx'$	$x'x'$	Total
Observed .....	2167	619	2786
Calculated .....	2089.5	696.5	2786
Difference (D).....	77.5	77.5	.....
Standard error (S. E.).....	22.85	.....	.....
D/S. E. ....	3.4	.....	.....

STATISTICAL STUDY OF STIPULE SHAPE IN THE  $F_3$  FAMILIES OF GRADUS ROGUE X MUMMY, AND RECIPROCAL

Besides classifying the  $F_3$  cultures on the basis of stipule character a number of families were chosen for a statistical study of the stipule shape. A record was kept of whether the families consisted of nonrogues only, rogues, or of both rogues and nonrogues. Table XX shows the frequency distributions and means of the cultures measured. In general the mean stipule ratio of each family tends toward the mean of the category to which the  $F_2$  parent belonged (Table XXI). Thus the means of the  $x'x'$  families tend to approach the mean of  $x'x'$  parent (Mummy) while the  $Xx'$  and  $XX$  families have means close to that of the  $XX$  parent (Gradus rogue). This tendency in the means of the three types of families is greater if the frequency distributions of the several families belonging to each group be combined. In Table XXII are shown the total frequency distributions from Table XXI of 28 segregating families, of 8 families breeding true to the rogue character, and of 15 families in which no rogues occurred. The mean of the rogue families is  $2.503 \pm 0.0093$  as compared with  $2.339 \pm 0.0096$  for Gradus rogue. The increased narrowness of the stipules of the rogue segregates over that of the rogue parent may be due to the accumulative effect of factors modifying stipule shape such as the Y and n factors responsible for stipule shape and stem fasciation, respectively, in Mummy. The mean of the segregating cultures is  $2.259 \pm 0.0062$  or very near the mean,  $2.333 \pm 0.0050$ , of all the  $F_2$  cultures of Gradus rogue  $\times$  Mummy. For the families in which no rogues appeared the mean is  $1.899 \pm 0.0053$  or intermediate between the means of Gradus type and Mummy,  $1.693 \pm 0.0107$  and  $2.088 \pm 0.0050$ , respectively. The mean of the  $x'x'$  families is especially significant as indicating a segregation into Graduslike plants and Mummylike plants among the  $F_3$  plants lacking the X factor. Such a segregation is to be expected in the progeny of  $F_2$  plants of the composition  $x'x'Yy$ , and from the effect of lumping together into one frequency distribution the  $F_3$  families with formulae  $x'x'YY$  and  $x'x'yy$ .

The evidence from the statistical study of stipule shape in the  $F_3$  families points to the homozygous nature of the broad or intermediate  $F_3$  segregates in regard to the  $x'$  factor. In addition the occurrence of  $F_3$  rogues homozygous and heterozygous for the X factor is also demonstrated by measurement of the stipules of their  $F_3$  progenies.

TABLE XXI.—Frequency distributions and statistical constants of ratio of (length of stipule) for the  $F_3$  families of *Grassus roque* × *Mummy*. The rogue families (indicated by R), the nonrogue families (indicated by N), and the families segregating into rogues and nonrogues (indicated by S), are marked in the extreme right-hand column.

Culture No.	Char- acters of $F_2$ percent.	1.45	1.35	1.25	1.15	1.05	0.95	0.85	0.75	0.65	0.55	0.45	0.35	0.25	0.15	0.05	0.00	Total.	M.	Coefficient variation.	Classi- fication of $F_3$ family.
1279.....	1.65	2	6	10	5	4	2	1										9	1.961±0.0245	5.60±0.8931	N
1281.....				11	9	7	3											29	1.792±0.0136	6.08±0.5332	N
1283.....				3	4	6												31	1.850±0.0186	5.62±0.4859	N
1285.....	1.75	3	3	4	7	4												13	1.873±0.0072	2.08±0.2649	N
1289.....		3	0	3	11	4												16	1.950±0.0178	5.43±0.6403	N
1293.....		3	2	3	2	3	4	1										25	1.950±0.0188	7.18±0.6884	N
1294.....		1	2	16	11	7	1	0										15	2.056±0.0315	9.09±1.0817	N
1301.....			1	1	2	5	15	4	3	1	2							60	1.759±0.0117	7.65±0.5069	N
1304.....				1	1	2	6	13	7	2	0	1	2					35	1.841±0.0247	11.17±0.9036	N
1306.....						5	6	4	7	5	3	7	2					32	2.050±0.0137	5.65±0.4776	N
1307.....						0	11	12	7	2	1	2						42	2.231±0.0247	10.67±0.7941	N
1309.....	1.85	1	1	0	0	0	11	12	7	2	1	2						37	2.050±0.0184	8.09±0.6393	N
1314.....						2	4	4	6	3	2	3	0	1	0	1		21	1.864±0.0035	1.28±0.3325	S
1320.....						2	4	4	6	3	2	3	0	1	0	1		12	2.089±0.0349	12.63±1.2000	S
1325.....						2	6	10	7	4								35	1.991±0.0133	8.99±1.2491	N
1341.....						2	6	16	11	7	1							12	1.901±0.0348	6.07±0.4854	N
1342.....	1.95					2	2	3	2	2	7	10	0	3	1	2		44	2.308±0.0262	11.17±0.8131	N
1343.....						2	2	3	2	2	7	10	0	3	1	2		16	2.331±0.0239	6.09±0.7179	S
1346.....						2	2	10	14	8	1	3	2	1	0	2	1	40	2.124±0.0239	11.34±0.8076	S
1347.....						1	7	7	5	2	2	1						25	1.990±0.0199	7.43±0.7127	S
1353.....	2.15					3	0	0	5	11	12	10	5	5	1	2		70	2.310±0.0179	9.54±0.5487	S
1418.....						3	3	8	4	4	6	5	3	1				37	2.117±0.0242	10.01±0.7919	S
1419.....						3	4	9	9	11	10	7	10	14	4	1	1	35	2.427±0.0140	6.34±0.4093	S
1423.....						1	1	1	4	5	4	3	7	3	2	1	1	64	2.071±0.0106	11.26±0.6786	S
1425.....						1	1	1	4	5	4	3	7	3	2	1	1	33	2.207±0.0328	12.68±0.6995	S
1428.....						3	5	8	4	1	0	1	0	1	0	2	1	25	2.010±0.0066	2.43±0.2319	S
1430.....						3	5	8	4	1	0	1	0	1	0	2	1	25	2.022±0.0308	11.32±1.0930	S
1445.....	2.35					1	6	6	9	9	1	3	1	4	2	0	0	45	2.132±0.0097	13.88±1.0057	S
1446.....						1	2	7	5	3	5	9	3	3	2	1	1	37	2.068±0.0248	10.69±0.8477	S
1450.....						3	3	5	9	4	3	2	1	0	2			10	2.138±0.0201	10.71±0.8741	S
1458.....						1	1	2	8	12	7	6	1	0	2			49	2.128±0.0168	8.22±0.5038	S
1467.....	2.45					1	1	2	8	2	7	4	7	3	3	1	1	38	2.105±0.0267	10.77±0.8429	S
1437.....											1	5	9	2	6	10	3	44	2.606±0.0220	8.32±0.6023	S
1438.....											2	0	1	3	3	5	2	16	2.362±0.0364	9.99±1.2041	S
1440.....	2.55										1	0	1	5	2	3	1	42	2.462±0.0147	5.76±0.4453	R
1441.....											1	1	1	3	1	0	1	14	2.164±0.0440	11.27±1.4548	R
1442.....											2	3	9	3	5	10	2	50	2.150±0.0594	14.58±1.0041	S

[illegible]

TABLE XXII.—The combined frequency distributions and derived statistical constants of ratio of  $\frac{\text{length of stubble}}{\text{width of stubble}}$  for the rogue (R), segregating (S), and the nonrogue (N) families of the  $F_3$  generation from cross Gradus rogue  $\times$  Mummy based on data in Table XXI.

Cultures. No.	Classi- fication.	1.45	1.55	1.65	1.75	1.85	2.05	2.15	2.25	2.35	2.45	2.55	2.65	2.75	2.85	2.95	3.05	3.15	3.25	3.35	Total.	M.	s.	Coefficient variation.	
8	R	...	...	...	...	...	4	17	27	40	51	57	27	16	12	3	1				258	2 501 ± .0003	0 2230 ± .0066	8.89 ± .0655	
28	S	1	1	13	47	84	120	119	119	121	108	73	61	41	29	12	8	3	1	4	1,045	2 249 ± .0302	-.306 ± .0044	11.54 ± .1011	
15	N	1	4	37	64	87	97	61	27	9	1										301	1,890 ± .0033	.154 ± .0037	8.81 ± .2141	
Total.....	.....	2	5	50	111	171	218	198	163	155	162	140	130	88	57	41	24	11	5	1	4	1,745			Σ

## DISCUSSION OF RESULTS.

True-breeding hybrids have been found in other genera than *Pisum*, notably in *Oenothera*, and have been interpreted by Muller (10) as due to the action of balanced lethal factors. In self-fertilized *Gradus* plants there is no evidence of zygotic lethals since under proper growing conditions all the ovules in a pod develop. Although an occasional sterile rogue is met with, all ovules generally form viable seed. In over 60 instances crosses between *Gradus* and *Gradus* rogue gave an average of 4.5 seeds per pod out of a possible 7 or 9. About 4 seeds per pollination is the number generally obtained from artificial crosses of *Gradus* with other varieties. However, it has been customary to pollinate two or three stigmas with the pollen of one flower, the result being one well filled pod and one or two partially filled, as a consequence of deficient pollination.

A microscopical examination of the pollen of *Gradus* and *Gradus* rogue showed well-developed pollen grains in both forms. Their pollen is about 100 per cent perfect. There was no morphological evidence of defective pollen which might be interpreted as due to the action of gametic lethals.

It is, therefore, apparently out of the question to explain the anomalous behavior of the rogues in crosses with *Gradus* types as due to the presence of zygotic or gametic lethals.

As previously stated, the nonappearance of *Gradus* segregates in the  $F_2$  generation of the cross *Gradus* with *Gradus* rogue is explained by Bateson and Pellew (1) as probably due to somatic segregation of the type and rogue "elements" in the  $F_1$  hybrid. The same authors have not suggested, however, a mechanism by which somatic segregation is accomplished. Such segregation would occur if somatic nondisjunction took place early in the development of the  $F_1$  plants, so that of the two daughter cells formed, one would receive both of the chromosomes derived from one member of the heterozygous chromosome pair present in the parent cell, and the other daughter cell would receive the two chromosomes derived from the second member of the heterozygous pair. As a result, both cells would retain the characteristic number of somatic chromosomes and at the same time be homozygous for either the type or rogue factors.

Besides assuming the phenomenon of somatic nondisjunction, the further assumption must be made that the daughter cells homozygous for the rogue factors increase more rapidly than cells containing the type factors, and consequently the  $F_1$  plant becomes more roguelike as it matures. At sporogenesis only rogue tissue would take part in gamete formation; therefore type plants would not appear among the  $F_2$  progeny.

Although somatic nondisjunction offers a hypothesis to account for somatic segregation it does not explain satisfactorily the failure of the type factors or "elements" to be present in the gametes of the  $F_1$  hybrids. Certainly accepted instances of somatic nondisjunction are rare, the best examples being the gynandromorphs in *Drosophila* described by Morgan and Bridges (9) and attributed to the effect of the dropping out of the sex chromosome at an early division of the fertilized egg. The demonstration of the loss of a chromosome was possible from a knowledge of the factors linked with sex. As there are no factors known which are linked to the type allelomorphs of the rogue factors, the loss, by nondisjunction in the  $F_1$  hybrid, of the chromosome carrying the type factor can not be detected. In addition, in the  $F_1$  plants nondisjunctional

nuclear divisions would have to take place too regularly and too often to make the explanation seem reasonable.

As an alternative hypothesis, the idea of "mass" somatic mutation is advanced to account for the anomalous behavior of the rogues in heredity. Using the factor symbols here adopted the  $x$  factor of Gradus is assumed to mutate occasionally to the  $X$  factor of Gradus rogue. The factor pair  $xx$  when in a homozygous condition is relatively stable and mutation to  $X$  rarely takes place. However, the heterozygous  $Xx$  combination is very unstable and  $x$  mutates with great frequency to  $X$ , creating a homozygous and more stable condition of the germplasm in regard to the  $X$  factor. Mass somatic mutation of  $x$  to  $X$  occurs in the soma of the  $F_1$  hybrid at an early stage in its development and would account for the prevailing absence of the  $x$  factor among the  $F_1$  gametes. The same phenomenon would be true of Gradus plants in which the primary mutation of  $x$  to  $X$  took place. Such a mutation occurring early in the ontogeny of a Gradus type plant would produce an individual which would gradually become more roguelike as it matured. Or when the mutation takes place late in the development of the plant, the form of the individual would not necessarily be changed but the issue derived from the cell containing the  $X$  factor, would, if it entered into gamete formation, cause rogues to appear in the next generation.

The fact that occasionally an  $F_1$  hybrid of Gradus  $\times$  Gradus rogue is Graduslike at maturity rather than roguelike is interpreted as indicating that rare Gradus gametes are produced, as a consequence of mutation or otherwise, in which the  $X$  factor is replaced by an allelomorph comparable in stability to the  $x'$  of Mummy.

It is probable that the rogues are an extreme manifestation of a series of mutations originating by changes in the  $x$  factor of Gradus. Thus the various sorts of intergrading intermediates described by Bateson and Pellew (1) may well represent various modifications of the factor  $x$  which may be designated as  $x''$ ,  $x'''$ , etc. The  $xx''$  or  $x''x''$  combinations produce intergrading intermediates in which mutation of either of  $x$  or  $x''$  to  $X$  is more frequent than in Gradus type. The differences in the stability of the various modified  $x$  factors as exhibited in the rate of change of these factors to  $X$ , accounts for the existence of high and low rogue-producing strains of intermediates.

The difference in the proportion of rogues produced from the lower as compared with the upper nodes of intergrading intermediates, besides being due to a multiplication of the somatic cells in which a primary mutation of  $x$  to  $X$  has occurred, may conceivably be caused by an increase in the number of somatic mutations brought about by physiological changes in the protoplasm as the plant matures.

The mutation of  $x$  to  $X$  in Gradus, in the  $F_1$  hybrid and in the intergrading intermediate is comparable to what Emerson (5) calls a recurring somatic mutation. The varieties in which rogues arise are of the sort earlier described by de Vries (13) as "ever-sporting," meaning that it is impossible to free the stock of the tendency to produce "sports" (mutations).

The results in the  $F_2$  generation of crossing Gradus type with Mummy and Gradus rogue with Mummy have led to the assumption of a factor pair  $Yy$  in addition to the  $xx$  factors as partly responsible for stipule shape in Gradus and Gradus rogue. In Gradus there are at least two factor pairs  $xx$  and  $yy$  determining the ratio of width to length of stipule.

The *y* factor in Gradus is linked with the *a* factor for white flowers; its allelomorph *Y* is linked with the *A* factor for colored flowers in Mummy. The proof of the presence of two factors for stipule shape in Gradus and Gradus rogue lies in the fact that no linkage exists between the *X* factor (the mutated form of the *x* factor of Gradus) in Gradus rogue and the *a* factor for white flowers as determined from an analysis of the  $F_2$  generation of the cross Gradus rogue with Mummy.

The allelomorph, in Mummy, of factor *x* is probably not identical with *x* but is very similar in its somatic expression. At least in combination with *y* of Gradus (the  $x'x'yy$  segregates from the  $F_2$  generation of Rogue  $\times$  Mummy), the  $x'x'yy$  plants have stipules very nearly like Gradus type. The factor allelomorph to *x* and found in Mummy is called  $x'$  to distinguish it from the *x* factor of Gradus. Additional evidence of a difference between *x* and  $x'$  is the fact that  $x'$  has never been known to mutate to *X* when  $x'$  is present in the homozygous state. The factor  $x'$  is apparently more stable than *x*.

In addition to the assumed inherent stability of the  $x'$  factor found in Mummy it is probable that the *Y* factor also acts as a stabilizer of the germ plasm. The heterozygous  $F_1$  plants of Gradus rogue  $\times$  Mummy ( $YyXx'$ ) have a germ plasm which is certainly less affected by the presence of the *X* factor than is the heterozygous  $Xxyy$  germ-plasm of Gradus rogue. With the  $Xx'$  combination, recurring somatic mass mutation to the extent obtaining in the  $F_1$  generation of Gradus type  $\times$  Gradus rogue, does not occur. The various factors that entered the zygote take part in gamete formation at maturity and give rise to the expected  $F_2$  combinations. Although *Y* behaves as a stabilizer of the  $F_1$  germ plasm and retards mass mutation of  $x'$  to *X*, the extent of the influence is difficult to determine. Nor is it known to what extent, if any, senility affects the relation of the *X* and  $x'$  factors. It is possible that, with increasing age, the  $F_1$  plants exhibit a change in the mutability of  $x'$  to *X*, resulting in an increase of rogues among the  $F_2$  segregates. Since other disturbing causes than somatic mutation in the  $F_1$  hybrid may combine to upset the expected number or ratio of  $F_2$  recombinations, the deviations of the observed number from the calculated number of variates in any one class can not be used, for the  $F_2$  data at hand, to approximate the rate of change of  $x'$  to *X*.

That changes in the *X* factor may occur in the  $F_1$  hybrids here discussed is indicated by the exceptional  $F_1$  plant (no. 9.727-6), which was similar in appearance and genetic behavior to the  $F_1$  hybrids of Gradus type  $\times$  Mummy. Additional evidence of mutation of *X* to some other form is shown in the behavior of two roguelike  $F_1$  plants (No. 9.722-1 and No. 9.722-5). These plants apparently produced very few, or no, rogues in the next generation (families 0.1059 and 0.1093). The primary mutation or mutations of *X* in the  $F_1$  parents was here delayed until late in development and, while not affecting the soma, radically changed the genetic character of the microspores and macrospores.

An analysis of the  $F_2$  generation of the cross Gradus rogue with Mummy substantiates the hypothesis of Mendelian inheritance of the rogue factors complicated by recurring somatic mutation. The number of segregating to nonsegregating  $F_2$  families is in close agreement with the theoretical number of 1  $XX$  : 2  $Xx'$   $F_2$  rogue segregates expected from a monohybrid  $F_1$  generation. The ratio of  $XX$  to  $Xx'$  plants in the progenies of heterozygous  $F_1$  rogues is disturbed by the phenomenon of recurring somatic mutation.

The discrepancy between observation and theory is great in respect to the ratio of rogues to nonrogues in the segregating  $F_3$  families. However, the fact of segregation is considered more important than the ratios obtained. The instability of the heterozygous  $Xx'$  combination, though not leading to a complete elimination of the  $x'$  gametes, by mutation of  $x'$  to  $X$ , is such as to produce an excess of  $X$  gametes over the number that would normally be formed if somatic mutation did not take place.

#### SUMMARY

(1) The Gradus variety of *Pisum sativum*, characterized by broad, wavy, emarginate stipules with a mean ratio of  $\frac{\text{length of stipule}}{\text{width of stipule}}$  of 1.70, occasionally produces rogue mutations, characterized by narrow, flat, pointed stipules with a mean ratio of 2.35.

(2) Primary rogues are produced from Gradus types by a mutation of a single factor  $x$  to  $X$ . They are therefore heterozygous, of the formula  $xX$ . They give an  $F_2$  of homozygous secondary rogues,  $XX$ , through recurrent (mass) somatic mutation of the  $x$  factor to  $X$ . Such recurrent mutation is believed to occur because of the instability of the combination of  $xX$ .

(3) The crosses Gradus  $\times$  Gradus rogue, and reciprocal, likewise produce an unstable  $F_1$  germ plasm in which mass, somatic mutation of  $x$  to  $X$  occurs.

(4) The effect of mass somatic mutation in the  $F_1$  hybrid is to produce a germ plasm homozygous for the  $X$  factor in by far the greater number of the somatic cells. At gametogenesis only an occasional  $x$  gamete is produced. The  $F_2$  generation consists almost entirely of rogues ( $XX$ ), the Gradus type ( $xx$ ) very rarely appearing.

(5) In addition to the  $x$  factor a second factor  $y$  for stipule shape is present in both Gradus and Gradus rogue. The factor  $y$  is linked with the  $a$  factor for white flowers.

(6) The English Mummy pea, a nonrogue producing variety, has a stipule ratio of 2.05 determined by a factor  $Y$ , allelomorphous to  $y$  of Gradus, and linked with the  $A$  factor for colored flowers.

(7) In crosses between Gradus and Mummy the number of cross-overs between  $AY$  and  $ay$  is approximately 20 per cent.

(8) Mummy, in addition to the  $Y$  factor, has a factor  $x'$  which is allelomorphous to the  $x$  factor in Gradus type, therefore to the  $X$  factor of Gradus rogue. The  $x'$  factor is practically identical with the  $x$  factor in its expression but differs in that it only rarely mutates to  $X$ , even in the combination  $x'X$ .

(9) The germ plasm of the  $F'$  cross, Gradus rogue ( $yyXX$ )  $\times$  Mummy ( $YYXx'$ ) is fairly stable. In the  $F'$  hybrids,  $YyXx'$ , the factor  $x'$  mutates to  $X$  but not to such an extent as to prevent at gametogenesis the formation of the usual gametes,  $XY$ ,  $Xy$ ,  $x'X$ , and  $x'y$ , as indicated by the appearance in the  $F_2$  generation of rogues (having the  $X$  factor), Graduslike plants (having the  $x'$  and  $y$  factors), and Mummylike segregates (having the  $x'$  and  $Y$  factors).

(10) The expected 3 : 1 ratio of rogues to nonrogues in the  $F_2$  and succeeding generations obtained from the heterozygous  $Xx'$  plants, is disturbed by an excess of  $X$  gametes and consequently of  $XX$  and  $Xx'$  zygotes.

(11) Inheritance of the factors for stipule shape  $X$  and  $y$  in Gradus rogue is Mendelian. Normal inheritance is obscured by somatic mutation of the factors  $x$  and  $x'$  when in the  $Xx$  or  $Xx'$  combination.



(12) In addition to determining a stipule ratio of 2.05 in Mummy, the factor Y acts as a stabilizer of the germ plasm. Somatic mutation of  $x'$  to X in the  $F_1$  cross between Mummy and Gradus rogue ( $Xx'Yy$ ) does not take place to the extent in which mutation of x to X occurs in the  $F_1$  crosses between Gradus type and rogue ( $XxYy$ ) on account of the presence of the Y factor.

(13) Besides mutations of x and  $x'$  to X, other mutations of x and  $x'$  occur which affect stipule shape. The nature of the latter kind of mutations has not yet been investigated.

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PLATE I

- A.—Upper part of a mature type plant of *Gradus*.  $\times \frac{1}{4}$ .  
B.—Upper part of a mature rogue plant of *Gradus*.  $\times \frac{1}{4}$ .





**PLATE 2**

**A** mature plant of the Mummy variety (P16).

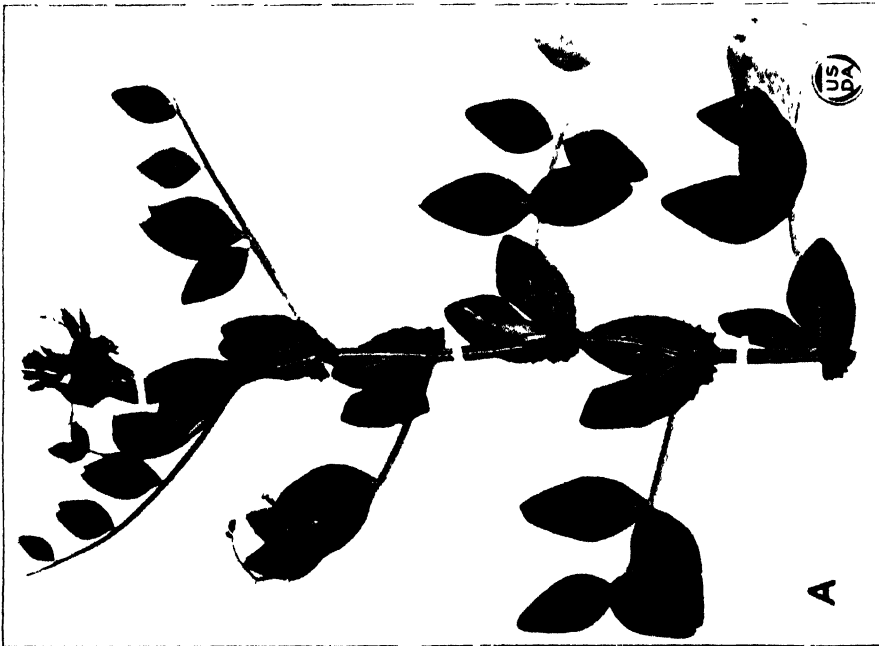
**A.**—The tip.  $\times \frac{1}{8}$ .

**B.**—The part immediately below the portion shown in A.  $\times \frac{1}{8}$ .

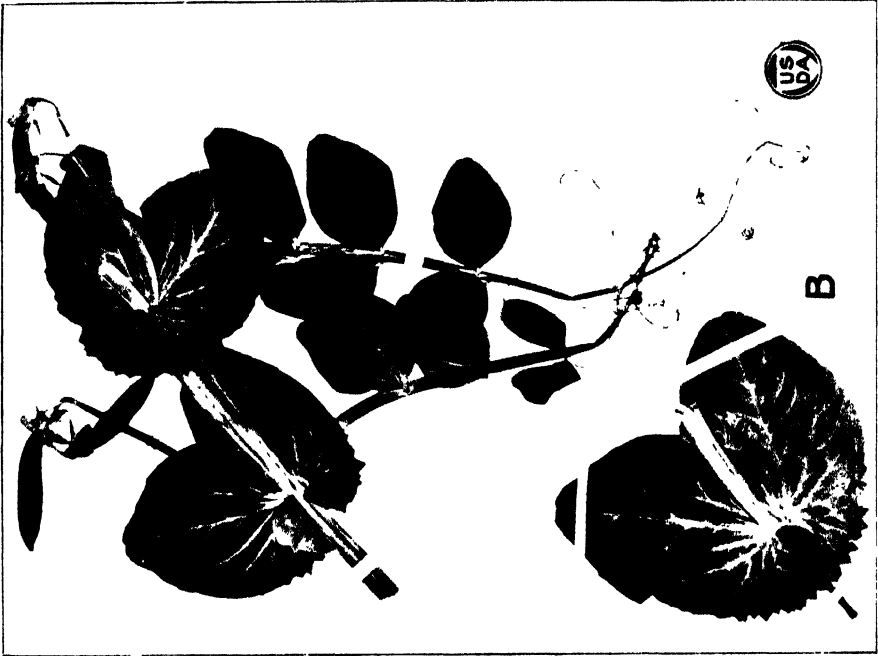
**C.**—Lower part of the stem.  $\times \frac{1}{8}$ .

**PLATE 3**

- A.—Upper part of a branch of a mature  $F_1$  plant of Gradus type  $\times$  Mummy.  $\times \frac{1}{4}$ .  
B.—Upper part of a branch of a mature  $F_1$  plant of Gradus rogue  $\times$  Mummy.  $\times \frac{1}{4}$ .







**PLATE 4**

A.—Upper part of a mature broad (y) segregate from the  $F_2$  generation of Gradus type  $\times$  Mummy. Note the typelike emarginate stipules.  $\times \frac{1}{4}$ .

B.—The part immediately below the portion shown in A.  $\times \frac{1}{4}$ .

PLATE 5 <sup>a</sup>

A.—Upper part of an intermediate (Y) or Mummylike segregate from the F<sub>2</sub> generation of Gradus type × Mummy. Note the narrow pointed stipules. × ¼.

B.—The part immediately below the portion shown in A. × ¼.

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<sup>a</sup> Plates 4 and 5 are of plants from the same F<sub>2</sub> family.



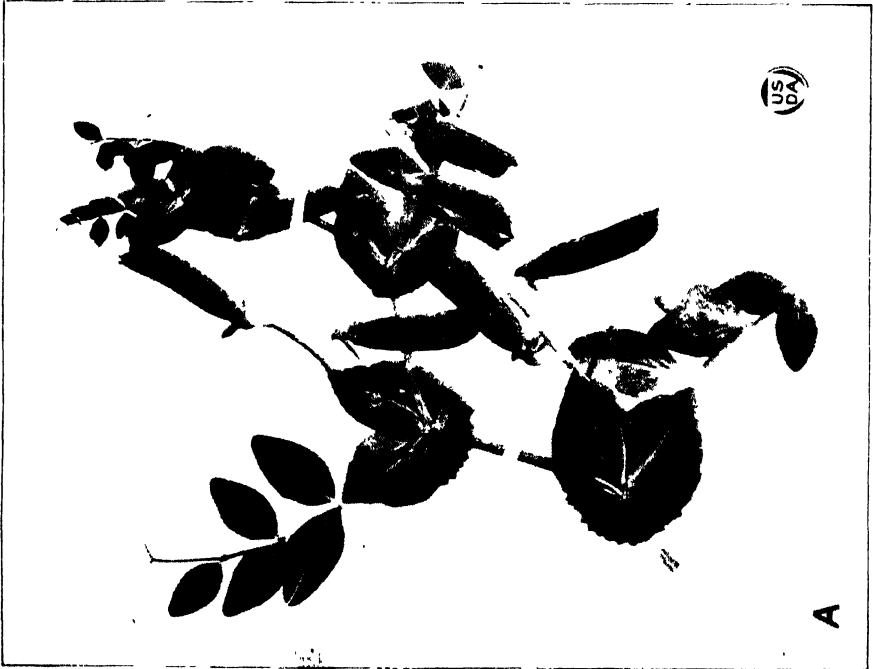


PLATE 6

A.—Upper part of a mature broad segregate from the  $F_2$  generation of Gradus  
rogue  $\times$  Mummy.  $\times \frac{1}{4}$ .

B.—The part immediately below the portion shown in A.  $\times \frac{1}{4}$ .

**PLATE 7**

**A.**—Upper part of a mature intermediate segregate from the  $F_2$  generation of *Gradus* rogue  $\times$  Mummy.  $\times \frac{1}{4}$ .

**B.**—The part immediately below the portion shown in A.  $\times \frac{1}{4}$ .





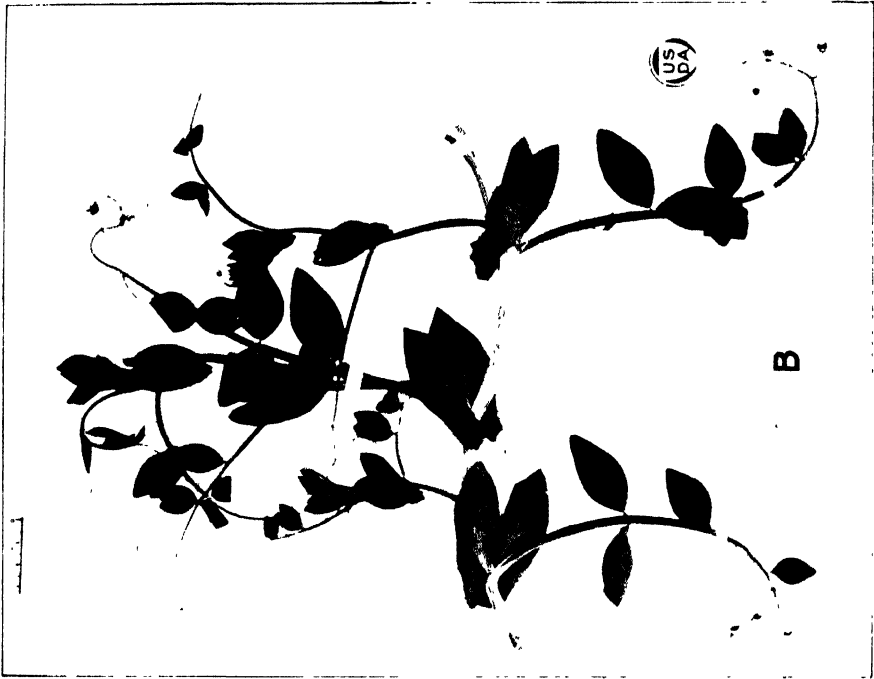


PLATE 8 <sup>b</sup>

A.—Upper part of a mature rogue segregate from the F<sub>2</sub> generation of Gradus  
rogue × Mummy. ×  $\frac{1}{4}$ .

B.—The part immediately below the portion shown in A. ×  $\frac{1}{3}$ .

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<sup>b</sup> Plates 6, 7, and 8 are of plants from the same F<sub>2</sub> family.



# A METHOD OF TREATING MAIZE SEED TO DESTROY ADHERENT SPORES OF DOWNY MILDEW<sup>1</sup>

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## POSSIBLE INTRODUCTION OF ORIENTAL DOWNY MILDEWS

Introduction into the United States of the *Sclerospora* downy mildews, which attack maize and related crops in the Orient, would be especially disastrous. These diseases are by nature essentially destructive and cause tremendous losses in the Orient. According to all available information they would prove equally destructive in the southern portion of our own corn belt.

Despite the dangerous possibility of thus introducing such diseases, there is a fundamental need of importing maize, not in commercial quantities, but in small quantities for experimental purposes. As the extensive maize breeding of Mr. G. N. Collins, of the Office of Crop Acclimatization and Adaptation Investigations, has shown, there may be obtained from the peculiar types of maize growing in remote countries valuable characters which can be combined most advantageously with those of our own varieties.

Under the Federal Horticultural Board regulations (including Quarantines 21, 24, and amendments), which thus far have prevented the importation of these mildews, the procedure of introducing foreign varieties of maize and related Gramineae from infested countries is necessarily difficult. As is requisite, such seed is inspected, given such treatment as any insect pest or disease found on it may demand, and grown under constant observation in an isolated quarantine greenhouse. Seed from healthy plants thus grown may then be planted without restriction by the experimenters to whom the original shipment was consigned. This procedure, although reasonable and necessary, is time-consuming and laborious.

A method of treating maize seed which will eliminate with absolute certainty any possibility of introducing maize mildews on such seed would be highly desirable. It is the purpose of this paper to present a method which the writer, after considerable experiment, has found to meet these requirements.

The oriental downy mildews of maize and related Gramineae all belong to the genus *Sclerospora* of the phycomycetous order Peronosporales. Several species are involved, but in the main features of structure and reproduction they agree. Through the work of Raciborski (9),<sup>2</sup> Rutgers (11), and Palm (8) on the Javan maize mildew; of Butler (2) on the maize mildew of India; of Lyon (5, 6), Miyake (7), and Lee (4) on the maize

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<sup>1</sup> Accepted for publication Nov. 24, 1922.

<sup>2</sup> Reference is made by numbers (italic) to "Literature cited," p. 859-860.

and sugar-cane mildew of Queensland, Fiji, Formosa, and the Philippine Islands; and of Reinking (10) and Weston (14, 15, 16) on the maize mildews of the Philippines, our knowledge of these forms is fairly complete. We know that if they should be carried to the United States on maize seed, it would be by resistant mycelium within the seeds, or by conidia or oospores on them. That mycelium should be the means is most improbable. Palm's work on the Javan maize mildew and unpublished experiments of the writer on the Philippine mildew of maize show that in the case of these two *Sclerosporas* at least, mycelium, although frequently penetrating the seed of badly infected ears, apparently is unable to transmit infection to the seedling developing therefrom. In such vegetative propagative parts as cuttings of sugar cane, living mycelium of *Sclerospora* may be carried and transmitted; but, in the case of maize, which is propagated only by seed, this difficulty is avoided.

Conidia adherent to maize seed can not accomplish infection. In the Philippine maize mildews the writer has found that conidia do not survive drying even for as short a time as one hour. Butler (2) in India and Miyake (7) in Formosa find this to be true of the maize mildews of those countries also.

It is preeminently probable that oospores on or in the maize seed can transmit infection. In the case of the closely related downy mildew of millet (*Sclerospora graminicola* [Sacc.] Schroet.) there is every evidence that intercontinental spread has been accomplished in this way. Whether the same means of distribution operates in the maize *Sclerosporas* we do not know. It should be noted in this connection that, although none of the several oriental mildews of maize has ever been found to produce oospores on maize itself, nevertheless these resting spores may occur abundantly on related hosts from which they can reach adjacent maize plantings.

Since the conidia are so easily killed, it will be by the resistant oospores, if at all, that these downy mildews will be introduced on maize seed. Consequently seed treatment must be directed against the oospores. To be effective in the case of *Sclerospora* diseases, a method of seed treatment not only must destroy any of these resistant spores which may be present but also must meet certain unusual requirements. The efficacy of the usual types of seed treatment is customarily demonstrated by the failure of adherent spores to give growth of the disease-producing fungus when the treated seeds have been placed in suitable media. But this would prove no index in the case of *Sclerospora*, as germination of the oospores has never been seen, and growth of the fungus on artificial media has never been secured. To be successful for *Sclerospora*, then, a method must give conclusive visible evidence of the destruction of the oospores.

#### EXPERIMENTS IN SEED TREATMENT

With this end in view, experiments were made with the substances customarily used in seed treatment. Of these, concentrated sulphuric acid fulfills the requirements most successfully, since it gives visible evidence of oospore destruction and yet does not impair seed germination. This was determined as follows:

Oospores (both fresh and two years dried) of the *Sclerospora* species so common (14, 15) on *Saccharum spontaneum* L. and *Miscanthus japonicus* (Thunb.) Anders. in the Philippines were mounted on a slide and carefully observed through the microscope while a drop of concentrated

sulphuric acid was drawn under the cover glass. Immediately the spores swelled and became distorted, either bursting at once or else less violently extruding oil drops and protoplasmic content while the wall softened and split open. Both content and wall rapidly darkened. Addition of India ink to the acid made more noticeable the extrusion of even small amounts of spore content. After five minutes all the spores in the field were obviously collapsed and exploded, or otherwise gave every evidence of being killed. The effect of first wetting the spores with alcohol was tried, a drop of alcohol being drawn under the cover of a dry mount, left about one minute, drawn off, and replaced by acid. In this case, the extrusion of the contents and the killing of the spores were even more rapid and complete. Water as a wetting agent was less effective than alcohol. There apparently was no difference between the wetting action of 80 to 95 per cent ethyl, methyl, or even denatured alcohol, or between the destructive effects of chemically pure or commercial concentrated sulphuric acid.

Experiments were next made to determine what effect this spore-destroying treatment would have on maize seed. Dry seeds of typical dent, flint, sweet, pop, and waxy-endosperm varieties of maize were put in separate glass jars, covered with concentrated sulphuric acid, and stirred occasionally with a glass rod during the 5 to 20 minutes of treatment. The acid was then drained off, and the seed washed for one hour in running water. Half of each lot of seed was planted, its germination recorded, and the character of the resulting plants observed. The other half was carefully dried, and its keeping quality tested by planting experimental lots from time to time. Although no quantitative results were obtained, it was found that even after the most severe treatment the viability was retained by a considerable percentage of the seed for as long a period as three months, even under the unfavorable rainy season conditions of the Philippines.

To prevent the persistence of minute air bubbles that might protect occasional spores from the acid, similar lots of seed were first given a preliminary wetting with alcohol for about a minute, the alcohol drained off, and the acid added. No difference either in immediate wetting effect, in subsequent action of acid on the seed, or in ultimate germination of the seed was apparent when 80 to 95 per cent ethyl, methyl, or denatured alcohol was used. Also there was no apparent difference between the effect of chemically pure or commercial concentrated sulphuric acid. As a result of the action of the acid, especially after treatments of 15 to 20 minutes, the surface of the seed became somewhat blackened, but the germination percentage was not decreased beyond practicable limits by even the longest treatments. Fresh acid was used for each lot of seed for fear that the black gelatinous material remaining in the acid might dilute it sufficiently to decrease its efficiency.

Some of these experiments in which representative kinds of maize were used are summarized in Table I. Calamba yellow flint is a typical example of the flinty types grown by the Christian Filipinos quite generally throughout the Philippines, Boone County White represents the American dent corn that has been introduced and successfully grown on large haciendas in several parts of the islands, while Manobo waxy is typical of the small, rapidly maturing varieties of maize cultivated by some of the non-Christian tribes in the mountains.

TABLE I.—Effect of treatments with sulphuric acid on germination and growth of three types of maize

Lot.	Treatment. <sup>a</sup>		Growth after 4 days.				Growth after 10 days.				Growth after 16 days.				Yield after 1½ months.					
	Num-ber of kernels.	Alcohol.	H <sub>2</sub> SO <sub>4</sub>	Planted, 1919.	Number of plants.			Number of plants.			Number of plants.			Number of plants.			Plants.	Ears.	Nub- bins.	Bar- ren.
					Total.	Nor- mal.	Abnor- mal. <sup>b</sup>	Total.	Normal.	Abnor- mal. <sup>b</sup>	Total.	Nor- mal.	Abnor- mal. <sup>b</sup>	Aver- age height, inches.	Num- ber of leaves.					
																Aver- age height, inches.				
1a	40	0	5	May 24	28	26	2	2-3	32	26	6	3-4	32	30	2	9	18	6	12	0
1b	40	½	5	do.	34	28	6	2-3	34	34	0	4-5	34	34	0	9	20	4	5	11
2a	40	0	10	do.	2	2	0	1½	4	2	2	3-4	4	4	0	6	2	1	0	1
2b	40	½	10	do.	28	16	12	1½	28	28	0	5	28	10	0	6½	22	10	12	0
3a	40	0	15	do.	32	26	6	1½	32	32	0	5½	32	32	0	9	15	3	7	5
3b	40	½	15	do.	20	14	6	1½	22	18	4	3-4	22	18	4	8	6	1	0	5
4a	40	0	20	do.	4	4	0	(c)	14	2	12	2½	14	2	12	5	7	1	3	3
4b	40	½	20	do.	1	1	0	1½	4	4	0	4	4	4	0	5	4	1	0	1
A	20	do.	do.	do.	20	20	0	2	20	20	0	6½	20	20	0	12	7	8	4	0
B	20	½	do.	do.	20	19	1	2	20	20	0	6½	20	20	0	12	10	7	2	1
C	20	1	do.	do.	20	17	3	1½	20	20	0	6	20	20	0	11	5	3	1	1
D	20	5	do.	do.	19	19	0	2	19	19	0	6	19	19	0	12	9	5	2	2
4c	40	0	20	May 28	12	8	4	1	16	10	6	4	16	10	6	3-4	15	12	2	1
4d	40	½	20	do.	22	12	10	1	22	18	4	3½	22	18	4	3-4	18	16	0	2
1c	10	0	5	Nov. 23	8	8	0	1	8	8	0	3½	8	8	0	3	6	3	0	3
1d	10	½	5	do.	7	4	3	1	6	3	d 3	4	6	3	3	4	4	2	2	0
2c	10	0	10	do.	6	6	0	1	6	5	1	3	6	5	1	3	4	2	1	1
2d	10	½	10	do.	8	8	0	1	8	8	0	3	8	8	0	3	6	1	1	4
BB	10	½	0	do.	0	0	0	1	8	8	0	1	8	8	0	3	7	2	3	2

CALAMBA YELLOW FLINT

## CALAMBA YELLOW FLINT

BOONE COUNTY WHITE DENT

	Ethyl, 85-95 per cent.	5 May 24.	24 16	20 8	4 8	1 1	2 2	26 18	24 10	2 2	4 1/2 4	3-4 3-4	26 18	24 10	2 2	6 6	4-5 4-5	11 8	8 4	3 3	0 0
1a	40	5	24	20	4	1	2	26	24	2	4 1/2	3-4	26	24	2	6	4-5	11	8	3	0
1b	40	5	16	8	8	1	2	18	10	2	4	3-4	18	10	2	6	4-5	8	4	4	0
2a	40	10	8	4	0	(c)	1	12	6	2	3 1/2	2-3	12	6	2	4 1/2	4	2	2	2	0
2b	40	10	4	4	0	1	1	6	6	2	3 1/2	2-3	6	6	0	4	4	2	2	0	0
3a	40	15	10	4	6	1	1	14	12	2	4	2-3	14	12	2	6	4-5	6	3	2	1
3b	40	15	4	4	0	1	1	8	8	0	4	3	8	8	0	6 1/2	4	5	0	3	2
4a	40	20	24	12	12	1	1-2	20	24	2	4 1/2	3-4	26	24	2	6	4-5	12	7	3	2
4b	40	20	30	14	16	1	1-2	28	26	2	4	3-4	28	28	0	6	4-5	15	6	0	3
A	20	do.	18	17	1	1 1/2	1-2	20	20	0	6 1/2	3-4	20	20	0	12	5-6	12	7	1	4
B	20	do.	20	20	0	1	1-2	20	18	0	6 1/2	3-4	20	20	0	12	5-6	13	4	7	7
C	20	do.	19	19	0	1	1-2	19	18	1	6	4	19	18	1	12	5-6	8	3	4	1
D	20	do.	19	19	0	1	1-2	19	19	0	6	4	19	19	0	12	5-6	7	2	1	4
		C. P. concentrated.																			
4c	40	20	20	16	4	1	1-2	22	20	2	3	3-4					14	8	1	5	6
4d	40	20	16	8	8	1	1-2	10	8	8	3	3-4					10	2	2		

MANOBO WAXY

	Dena- tured, full strength.	Commer- cial concentrated.	12 6	20 2	6 4	1 1	1-2 1	6 4	2 2	6 4	2 2	2-3 3	4 3	11 11	3 2	0 1	0 1	0 2
3b	40	15	do.	12	6	1	1-2	6	6	6	2	2-3	4	1	3	0	0	0
4b	40	20	do.	6	4	1	1	4	4	4	2	3	3	3	2	1	0	0
B	20	0	do.	19	19	0	1 1/2	19	19	19	5	4-5	13	11	2	2	0	0

a After treatment all lots were washed in running water for one hour and were then planted immediately.  
b Under "abnormal" plants are included those showing distortion, discoloration, or other evidences of atypical growth. It should be noted, however, that such growth results not only from chemical injury, as from acid, but also from mechanical injury, from flooding, or from attack by ants or other insects.  
c Plants just emerging at end of 4-day period.  
d One abnormal plant died.  
e Three abnormal plants died.  
f Two abnormal plants died.



Table I is significant only to the extent of showing that the method gives practical yields even after treatment more than rigorous enough to insure destruction of adherent spores. Too many extraneous factors are involved to allow significant comparison of such points as percentage of germination after different treatments or for different varieties. For example, the dryness and age of the seed were not in all cases the same, the location of the plots did not insure equal pollination, and the season of May to September, 1919, when most of the trials were made, terminated in six weeks of successive and violent typhoons that beat down and destroyed many plants.

From a large number of such experiments the following routine for the treatment of maize seed was formulated.

#### METHOD OF TREATING MAIZE SEED TO DESTROY ADHERENT SCLEROSPORA SPORES

- (1) Wet the seed with alcohol for one half to one minute; drain, and, while the seed surface is still damp—
- (2) Cover with concentrated sulphuric acid, allow to remain 5 to 10 minutes, stirring from time to time, then drain off the acid.
- (3) Wash for one hour in running water, stirring the seed occasionally to insure complete removal of the acid.
- (4) Plant at once, or dry thoroughly and save for future planting.

After carefully trying all phases of this method of treatment the writer is convinced that it meets successfully the requirements of the situation earlier outlined in this paper. By it oospores of *Sclerospora* that may be adhering to the maize seed may be destroyed, while at the same time the vitality of the seed is but little impaired. If planted at once, the percentage of germination is only slightly reduced, and most of the resulting plants are normal in growth and seed production. If dried and preserved, the percentage of germination declines gradually, but even after three months a sufficient number of normal plants will result to meet all practical purposes.

Preliminary experiments indicate that this method of treatment is equally effective against spores of the *Physoderma* disease of maize, which in the Orient involves two causal species (3, p. 114; 12, p. 245-247), one of them already introduced and destructive in the southern United States (13). Preliminary experiments have shown also that this treatment can be used successfully in the case of teosinte (*Euchlaena luxurians* Schrad.), coix (*Coix lachryma-jobi* L.), and some varieties of sorghum (*Andropogon sorghum* [L.] Brot.).

In its essential feature, i. e., the use of concentrated sulphuric acid, this method is by no means new. This reagent has been employed by several investigators for sterilizing the surface of many different varieties of seed. Brigham (1), for example, has used sulphuric acid to free popcorn seeds from mold spores customarily adherent to them; and the same reagent is used by the Federal Horticultural Board inspectors to destroy anthracnose spores on cottonseed. As modified to meet the exacting requirements of maize seed suspected of carrying *Sclerospora*, however, the method, it is hoped, will fill a peculiar need, enabling experimental quantities of seed of desirable varieties to be imported from quarantined regions afflicted with *Sclerospora* mildews, and insuring that, after treatment, the seed may be planted immediately without restriction and without danger.

## SUMMARY

In various parts of the Orient there are several downy mildews (*Sclerospora* spp.) that cause severely destructive diseases of maize. Yet it is desirable that seed of varieties with useful characters be imported even from such regions for experiment and breeding. This involves the danger of introducing these dreaded diseases into our own country by oospores adhering to the maize seed. Therefore such importations are grown in quarantine greenhouses to produce seed under constant observation, a costly and time-consuming procedure. There is need of some method of seed treatment which with absolute certainty will destroy any oospores which may be adherent, and make possible immediate and unrestricted planting of the seed. Such a method not only must give conclusive visible evidence of destroying\* the resistant *Sclerospora* oospores which might be on the seed, but also must leave the viability of the seed unimpaired. The writer finds that treatment with sulphuric acid after preliminary wetting with alcohol successfully fulfills these requirements. Details of the method are given in this paper.

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# INFLUENCE OF THE SUBSTRATE AND ITS HYDROGEN-ION CONCENTRATION ON PECTINASE PRODUCTION <sup>1</sup>

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## INTRODUCTION

Former investigations by the authors (9)<sup>2</sup> have shown that *Rhizopus tritici* Saito produces a substance of the nature of an enzym which has the power to dissolve the middle lamellae of raw sweet-potato (*Ipomoea batatas*) disks. This macerating principle was found to be thermolabile and to be produced abundantly in sweet-potato decoction in cultures one or two days old. It was found that while the mycelium itself retained some of the enzym which is called pectinase in these investigations, a portion of it was excreted into the culture solution on which it grew. Investigations have also proved that pectinase is produced by a number of other species of *Rhizopus*, some of which are able to cause a typical softrot of sweet potatoes. The amount of enzym produced was not equal in all cases. The most parasitic species did not necessarily produce the largest amount of enzym under cultural conditions. For example, *Rhizopus nigricans* Ehrb., which is the common softrot-producing organism in sweet-potato storage houses, produced a small amount of enzym in culture.

In order to study the action of the enzym on raw sweet-potato disks, the organism was grown for two or three days in a solution of sweet-potato decoction. The mycelium was then removed and treated according to a method described elsewhere (9). The ability of the enzym, contained both in the mycelium and in the solution, to macerate raw disks was tested. Measured portions of the solution were pipetted into small flasks, some of which were steamed to inactivate the enzym. Raw sweet-potato disks 1 cm. in diameter and 1 mm. in thickness were dropped into the steamed and unsteamed solutions which were then held at 45° C. Maceration was usually completed in from 2 to 4 hours in the solution containing the active enzym. The disks in the steamed controls had not been acted upon in that length of time. If, however, the disks in the control solutions were examined at the end of 24 hours, they were frequently found partially or completely macerated, the cells separating along the line of the middle lamellae in a manner typical of those in active enzym solution. In searching for an answer to this curious phenomenon two possible explanations presented themselves; first, that the enzym was not actually completely inactivated, although the solutions were steamed in an Arnold sterilizer for 10 minutes; second, that some other substance was produced which acted in a manner identical with the enzym itself.

When a modified Czapek's nutrient solution was used, a solution employed quite generally in these investigations, the results were even

<sup>1</sup> Accepted for publication Jan. 22, 1923.

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 877-878.

more striking. It was found that in the case of this solution steaming caused no change, the steamed solutions macerating as readily as the unsteamed. These results indicated that the enzyme was not secreted, but that some other substance was produced which had the power to dissolve the middle lamellae.

These results indicated two possibilities; first, that the enzyme was not secreted when the fungus was grown on certain substrates or only in a small amount; and, second, that although the enzyme was not secreted the organisms made the solution sufficiently acid to cause maceration of raw sweet-potato disks. In view of these facts, the authors undertook to make a study of the influence of the substrate on the production of pectinase, and of the changes in acidity produced by the fungus in different media as measured in terms of hydrogen-ion concentration. It will be shown in the discussion later that investigations have enabled the authors to explain some of the curious and puzzling results with respect to the parasitism of some of the species of *Rhizopus* causing soft-rot of the sweet potato.

Since *Rhizopus tritici* has been employed in so many of the investigations by the writers in this particular field, it was decided to use it in these studies. *Rhizopus tritici* grows readily in culture. It is parasitic on the sweet potato and produces an abundance of pectinase. Although not as common in storage as *Rhizopus nigricans*, it lends itself to physiological studies herein outlined more readily than does the latter species.

## INFLUENCE OF THE SUBSTRATE ON PECTINASE PRODUCTION

### METHODS OF EXPERIMENTATION

In order to determine what influence the substrate had on the production of pectinase, 10 different media were employed as follows: Bean, prune, Irish-potato, carrot, turnip, and sweet-potato decoctions, beef bouillon and a modified Czapek's, Pfeffer's and Richard's solutions. The vegetable and fruit decoctions were prepared by using 500 gm. of the fruit or vegetable in 1 liter of water, cooking for one hour and then filtering. Measured portions of these decoctions were put in small flasks, which were then plugged and autoclaved for about 20 minutes at 15 pounds pressure. The beef bouillon was prepared according to the usual method for preparing this medium. Some modifications were made in Czapek's, Pfeffer's, and Richard's solutions. For example, the  $\text{NaNO}_3$  in Czapek's solution was replaced by an equal amount of  $\text{NH}_4\text{NO}_3$ . Glucose, 50 gm. per liter, was used as a source of carbon. The substitution of  $\text{NH}_4\text{NO}_3$  for  $\text{NaNO}_3$  was made because previous experiments showed that *Rhizopus tritici* thrived better when the nitrogen was derived from ammonium nitrate. Investigations have also shown that the fungus can not utilize cane sugar, hence glucose was substituted for it.

Pfeffer's and Richard's solutions were made according to the following formulae:

	PEFFER'S.	RICHARD'S.
$\text{KH}_2\text{PO}_4$ .....	5.0 gm.	0.5 gm.
$\text{KNO}_3$ .....	.....	4.0 gm.
$\text{MgSO}_4$ .....	2.5 gm.	0.5 gm.
$\text{NH}_4\text{NO}_3$ .....	10.0 gm.	10.0 gm.
$\text{FeSO}_4$ .....	Trace.	Trace
Glucose.....	100 gm.	60.0 gm.
Water.....	1,000 cc.	1,000 cc.

The original Pfeffer's and Richard's solutions call for cane sugar, but, in view of the fact that the fungus used in these investigations does not utilize it, glucose was employed instead. All the different media were prepared in large quantities and held in flasks stoppered with cotton and covered with oiled paper until ready for use. Just previous to using, 30 cc. of the solutions were pipetted into each of fifteen 100 cc. Erlenmeyer flasks which were then autoclaved for 20 minutes at 15 pounds pressure. Ten flasks of each solution (set) were inoculated after which they were incubated at 35° C. The remaining 5 flasks of each set were held as controls.

At the end of the growth period, usually three to four days, the mycelium from all the flasks of a single series was collected into one compound sample and prepared for macerating experiments, according to methods previously described. The solutions on which the fungus grew in each set were made into one compound sample and equal portions (about 30 cc.) pipetted into small flasks to which raw sweet-potato and carrot disks were added. A portion of each of the solutions was steamed for 10 minutes to inactivate the enzym which, together with flasks of the original solutions which had not supported a fungous growth, were used as controls. An accurately weighed amount of mycelium (0.25 gm.) ground in pure quartz sand was used in determining the macerating action of the hyphae. The mycelium was not extracted in water prior to the addition of the raw disks, since previous experiments showed (9) that the rate of maceration was not influenced thereby. The ground mycelium was included in the system.

The hydrogen-ion concentrations of the solutions and of the controls (not inoculated) were determined by the electrometric method as rapidly as possible after the hyphae were removed, in all cases during the same day.

#### EXPERIMENTAL DATA

In this series of experiments 10 different solutions were employed, 6 being of vegetable origin, 3 synthetic solutions, and 1 of beef bouillon. In one or two series of experiments in which a modified Czapek's nutrient solution was employed as a substrate it was noted that no pectinase seemed to be produced, although it was abundantly secreted in sweet-potato decoction with which it was being compared. It was also noted in these cases that even in the absence of pectinase a certain amount of maceration of sweet-potato disks occurred. In view of these facts, experiments were undertaken to study the regulating action of certain substrates on the production of the enzym. The substrates here employed were selected as being representative of the vegetable decoctions and synthetic solutions in common use. It was also believed that if the substrate exercised any influence on the production of pectinase that fact would be brought out by these media.

#### MYCELIAL GROWTH

In these series of experiments the dry weight of the mycelium was not determined. The mycelium was all used in maceration experiments. In general, the growth and fruiting of the fungus on the media of vegetable origin were good, being much better than they were when the fungus was grown on the synthetic solutions or on beef bouillon. Bean, carrot, and turnip decoction yielded the best growth and fruiting. A fair growth took place in prune decoction, but little or no fruiting. The

growth and fruiting on Irish-potato decoction were good but somewhat inferior to what they were on bean, carrot or turnip. The growth was poorer on sweet-potato decoction than on any of the vegetable preparations. The growth on the three synthetic media and on beef bouillon was not as good as on the vegetable decoctions. A better growth was made on beef bouillon than on Czapek's, Pfeffer's, or Richard's solutions. The poor growth on Czapek's solution was rather surprising in view of the fact that *Rhizopus* usually grows well and fruits abundantly on it. The time for terminating the experiment was gaged by the stage of fruiting of the fungus. It was shown by Brown (2) and by Harter and Weimer (9) that the maximum amount of the macerating principle was contained in the mycelium just at or just preceding fruiting. Fruiting began a little earlier on the vegetable media than on the synthetic ones, consequently the experiments in which the vegetable media were used were terminated usually one day sooner than the others.

#### HYDROGEN-ION CONCENTRATION

The hydrogen-ion concentrations of the uninoculated solutions and the solutions on which the fungus grew are shown by Table I.

TABLE I.—*Hydrogen-ion concentration of inoculated solutions and controls (uninoculated at the end of the experiments in terms of  $P_H$ )*

Media.	Experiment 1.		Experiment 2.	
	Control.	Inoculated.	Control.	Inoculated.
String-bean decoction .....	4.88	7.82	4.80	7.99
Prune decoction .....	3.95	3.58	3.91	3.53
Irish-potato decoction .....	5.68	7.48	5.60	7.80
Carrot decoction .....	5.18	4.36	4.98	4.47
Turnip decoction .....	5.05	4.03	4.83	4.26
Sweet-potato decoction .....	5.22	3.24	5.05	3.31
Czapek's solution .....	4.84	2.34	4.14	2.47
Pfeffer's solution .....	3.53	2.56	3.48	2.61
Richard's solution .....	3.44	2.51	3.36	2.46
Beef bouillon .....	8.29	8.18	8.04	8.52

A survey of Table I shows that there is about as close an agreement in  $P_H$  values between the two experiments as could be expected from two experiments carried out at different times and under somewhat different conditions. The greatest variation noted is in the controls in connection with Czapek's nutrient solution. The solutions for the two experiments were prepared at one time so that those used for the second experiment stood for some time longer. A certain amount of evaporation would probably take place and chemical reactions are not unlikely.

A further examination of the table reveals other curious facts with respect to the hydrogen-ion concentration. The growth of the fungus caused some change in the hydrogen-ion concentration of all the solutions although in the case of beef bouillon and prune decoction it was not large. On the other hand, the change produced by the fungus when growing on string bean and Irish potato decoction and on Czapek's solution was quite marked. In experiment 1 in string-bean decoction the hydrogen-ion concentration was decreased from  $P_H$  4.88 to 7.82 and

on Irish-potato decoction from  $P_H$  5.68 to 7.48. The hydrogen-ion concentration was increased from  $P_H$  4.84 to 2.34 on Czapek's solution.

If a comparison is made of the hydrogen-ion change produced in the six vegetable decoctions, it will be seen that in some cases it is increased and in others decreased. This is contrary to what might be expected on theoretical grounds. Before these experiments were conducted it had been many times demonstrated that *Rhizopus tritici* made sweet-potato decoction more acid. For this reason it was predicted that it would produce similar changes when growing on other vegetable decoctions prepared in a similar manner. However, it was found that some of the decoctions (carrot, turnip, sweet potato) became more acid while others (string bean, Irish potato) became less so.

The synthetic media were all more acid at the close than at the beginning of the experiment, Czapek's nutrient solution showing a greater increase in hydrogen-ion concentration than either Pfeffer's or Richard's solutions. Beef bouillon, a solution not well suited to the growth of *Rhizopus tritici*, was very little changed in acidity, becoming less alkaline and more alkaline in experiments 1 and 2 respectively.

The outstanding conclusion to be drawn from these experiments alone is that it is not safe to class any one fungus as an acid or an alkaline-producing organism. These experiments too clearly demonstrate that an organism may be an acid producer on one medium and the reverse on another. The class to which an organism belongs would clearly seem to depend upon the media used and should be determined in each individual case.

#### ENZYM PRODUCTION

The amount of enzym produced was measured by the time required to macerate raw sweet-potato and carrot disks 1 mm. thick suspended in the solution on which the fungus grew and in a water suspension of the mycelium. The time required to effect loss of cohesion of the cells of disks by the enzym secreted into the solution of the different media and retained by the mycelium is shown by Table II. It must be remembered that these results are only approximate, since it is not always easy to tell just when loss of coherence is complete. It is believed, however, that they are accurate within the limits of  $\pm 5$  per cent.

TABLE II.—The time (in hours) required to macerate raw sweet-potato and carrot disks by the enzym in the solution and in a water suspension of the mycelium

Media.	Experiment 1.			Experiment 2.		
	Solution (sweet potato).	Hyphae.		Solution.		Hyphae (sweet potato).
		Sweet potato.	Carrot.	Sweet potato.	Carrot.	
String-bean decoction....	6.00.....	7.0.....	7 to 24.....	4.5.....	7 to 24.....	7 to 24
Prune decoction.....	None in 24.	7 to 24.....	.....	None in 24	None in 24.	7 to 24.
Irish-potato decoction....	3.75.....	5.0.....	7 to 24.....	3.0.....	6.5.....	7 to 24.
Carrot decoction.....	2.75.....	4.5.....	7 to 24.....	3.0.....	3.0.....	5 0
Turnip decoction.....	2.25.....	4.0.....	5.....	2.0.....	2.0.....	3.0
Sweet-potato decoction....	2.75.....	3.5.....	7 to 24.....	3.0.....	5.5.....	6.75.
Czapek's solution.....	None in 24.	Consider- able in 30.	None in 30.	None in 24	None in 24.	None in 24.
Pfeffer's solution.....	..do.....	..do.....	..do.....	..do.....	..do.....	Do.
Richard's solution.....	..do.....	..do.....	..do.....	..do.....	..do.....	Do.
Beef bouillon.....	..do.....	..do.....	..do.....	..do.....	..do.....	Do.



It was demonstrated that a macerating enzyme was produced by *Rhizopus tritici* when grown on all the vegetable decoctions, with the possible exception of the prune, and that this enzyme was quite active in some cases (carrot, sweet potato, and turnip). An examination of experiment 2, Table II, shows that loss of coherence of both sweet-potato and carrot disks was complete in the turnip decoction in two hours and in the carrot decoction in three hours, and in the suspension of hyphae grown on these decoctions in three and five hours respectively. The rate of maceration by the enzyme in the sweet-potato decoction and by that in the hyphae grown on this solution was about the same as that obtained in many previous tests. Although there was a fair growth of mycelium on prune decoction, the writers were unable to demonstrate the presence to any extent of pectinase either in the solution or in the hyphae. With this exception, the vegetable media all produced a demonstrable amount of pectinase. There was considerable variation in the amount produced in the different media, the complete loss of coherence being shown by some solutions in less than one-half the time required by others.

The production of pectinase could not be demonstrated in any of the synthetic solutions or in beef bouillon or the mycelium grown on them. From these results it seems safe to conclude that there is something in the composition of most of the vegetable decoctions which stimulates the production of pectinase, which is not present in the synthetic media or in beef bouillon.

A probable explanation of the difference between the synthetic media and the vegetable decoctions with respect to the production of pectinase by the fungus growing on them may be sought in the regulatory action of the substrate. A number of investigators have shown a quantitative regulation of the production of enzymes, while Knudson (15) demonstrated a qualitative regulation of tannase with *Aspergillus niger* and *Penicillium* sp. He found that these fungi produced gallic acid by the fermentation of tannic acid when the latter was added to a modified Czapek's nutrient solution, but if supplemented with glucose no tannase was formed. A number of other substances when used as a source of carbon failed to stimulate the secretion of tannase. Katz (14) studied the regulatory action of certain chemical substances in the substrate on the secretion of amylase by *Penicillium glaucum*, *Aspergillus niger*, and *Bacillus megatherium* and found that while the amylase secretion was not prohibited by the presence of substances chemically allied to starch, their effect was to greatly inhibit it. He found that the different fungi did not act identically and cites as proof the results obtained with *A. niger* and *P. glaucum*, in which case sugar had a much less inhibiting effect on the production of the enzyme with the former than with the latter. Similar conclusions were reached by Duclaux (6) with *P. glaucum* and *A. glaucus*, though he considered only the enzyme excreted into the culture medium. A number of other investigators, among them Kylin (16), have made similar studies. He worked with *P. glaucum*, *P. bifforme*, and *A. niger*, and found no qualitative regulation of the enzymes (diastase, invertase, and maltase), though a quantitative regulation was conclusively proved. In the case of *P. glaucum* the regulatory secretion of diastase was greater than in that of *A. niger*. In this same connection the results obtained by Young (26) with *A. niger* may be cited. He showed that inulase was produced in greatest amount in the mycelium when inulin was used as the source

of carbon, but was likewise produced when other carbohydrates were employed. The substances most closely allied to inulin were most efficient in the production of the enzyme. Investigations along similar lines have been made by Went (22), Wortmann (24), Dox (5), Pfeffer (19), Brunton and MacFayden (3), Harter (8), and others. Went, for instance, showed that *Monilia sitophila* secreted a number of enzymes, some of which were produced only when the particular substances on which they act were present in the culture solution. Brunton and MacFayden found that a bacterium formed diastase when cultivated on starch paste but not when grown on meat broth. Dox, on the other hand, demonstrated that the enzymes were secreted by *Penicillium camemberti*, regardless of the chemical nature of the substrate. He found that by cultivating the fungus on any particular substratum the quantity of the corresponding enzyme could be increased, but that no enzyme not normally produced by the organism could be developed by any special method of nutrition. Harter likewise showed that when different carbohydrates were used alone or in combination in the culture medium, although amylase was produced when sugars alone were employed, it was secreted in greatest abundance when starch was the only source of energy.

The preceding review of some of the literature shows that the quantitative regulation of enzymes is a rather common phenomenon. The qualitative regulation, however, has been demonstrated in only a few cases. Failure to demonstrate the presence of an enzyme does not necessarily constitute positive proof that it is not secreted. It is a well known fact that some enzymes act only under certain conditions—that is, in the presence of certain acids or alkalies or electrolytes or other substances, the so-called co-enzymes. For example, it has been shown that the presence of either the chlorin or bromin ion is absolutely essential to the activity of pancreatic amylase (1). It is a fact, however, that the writers have been unable to demonstrate the production of pectinase by *R. tritici* on Czapek's, Pfeffer's, and Richard's solutions and on beef bouillon. On the other hand, it was freely produced in all vegetable decoctions with the exception of prune decoction, where its secretion was doubtful.

With these facts in mind the writers suspected that there might be some substance or substances in the vegetable decoctions which were stimulating the production of pectinase that were absent in the synthetic media and in beef bouillon and that this substance was probably one or more of the pectic compounds. Experiments were therefore initiated in which pectin obtained in as pure a state as it was possible to make it was introduced alone and in combination with glucose into Czapek's modified nutrient solution as the available sources of carbon.

The pectin was obtained from the carrot, the method described by Hunt (10) being followed for the most part in its preparation. A number of flasks were prepared, using Czapek's modified nutrient solution as the substrate. In some cases dextrose (20 per cent), in others pectin (1 per cent), and in still others dextrose (20 per cent), and pectin (1 per cent) in combination were supplied as the source of carbon. Thirty cubic centimeters of these solutions were placed in each flask and after inoculation with a spore suspension of *Rhizopus tritici* the cultures were incubated at 35° C. At the end of 4 days' growth the mycelial felts from all of the flasks of a single series were combined into one compound sample and prepared according to the usual method with acetone and ether for macerating experiments. Likewise, the solutions on which the

fungus grew from a single experiment were made into one sample and immediately used for the maceration of raw sweet-potato disks. A portion of this solution which was first steamed to inactivate the enzyme together with the uninoculated solution, served as controls. Hydrogen-ion determinations were made of the inoculated solutions and of the uninoculated controls at the close of the experiment.

A good growth was obtained in each of the different series. The best growth and fruiting, however, were obtained when dextrose and pectin were combined in the same solution.

Some interesting results with respect to the hydrogen-ion concentrations were likewise obtained. In one experiment Czapek's solution plus 20 per cent dextrose with an original hydrogen-ion concentration of  $P_H$  5.24 had a  $P_H$  of 1.93 after the fungus had grown on it for 4 days. When 1 per cent pectin alone was substituted for the dextrose the solution had an original  $P_H$  of 4.38 and (after the fungus had grown upon it) a final hydrogen-ion concentration of  $P_H$  3.76.

In another experiment the following results were obtained: Czapek's solution plus 20 per cent dextrose with an initial  $P_H$  of 4.94 had a final  $P_H$  of 1.85. When 20 per cent dextrose and 1 per cent pectin were used the initial  $P_H$  was 4.09 and the final 1.70. If, on the other hand, pectin alone was used the original hydrogen-ion concentration was  $P_H$  4.43 and the final  $P_H$  3.90. The results show clearly that the pectin itself while supporting a good growth of mycelium is not so efficacious in the production of acid as dextrose.

Equally striking results were obtained with respect to the production of a middle lamellae dissolving enzyme. It has already been pointed out that pectinase was not produced on Czapek's nutrient solution with dextrose as a source of carbon. Comparative experiments as detailed above showed that only a feeble macerating principle could be demonstrated either in the mycelium or in the solution on which the fungus grew when Czapek's solution plus 20 per cent dextrose was used or when the same solution plus 20 per cent dextrose plus 1 per cent pectin were employed as the source of energy. On the other hand, when pectin alone was used as a source of carbon a vigorous cell wall dissolving enzyme was secreted. In one series of experiments coherence of the cells of sweet-potato disks immersed in the solution was entirely lost in  $3\frac{1}{4}$  hours when pectin alone was used. When dextrose was employed 24 hours were required. There was no maceration in 24 hours in the steamed control when pectin was used. When glucose was used maceration was completed in 24 hours, which was probably caused by the acid ( $P_H$  1.93) in the solution.

A second series of experiments gave results similar to those discussed above. When pectin alone was used as the source of carbon maceration in the solution and by means of the mycelium was completed in two and three hours, respectively. On the other hand, when glucose alone or in combination with pectin was employed 22 hours were required for the complete loss of coherence of the cells in the solution and in a water suspension of the mycelium. It is interesting to note in this connection that maceration was completed in the steamed controls in 22 hours, which may have been due to the acid ( $P_H$  1.85 in 20 per cent dextrose and  $P_H$  1.70 in 20 per cent dextrose + 1 per cent pectin) formed.

The results of the experiments as detailed above seem to indicate clearly a rather marked quantitative if not qualitative regulation of

pectinase. Although the qualitative regulation of pectinase has been demonstrated here for the first time, it is a well-known phenomenon for other enzymes. A brief review of some of the literature bearing on other enzymes showed that some of them, diastase for instance, are produced more abundantly when starch forms the only available source of carbohydrates in the solution.

#### INFLUENCE OF THE HYDROGEN-ION CONCENTRATION OF THE SUBSTRATE ON PECTINASE PRODUCTION

##### METHODS OF EXPERIMENTATION

Two different solutions, sweet-potato decoction and Czapek's modified solution, were used in the investigations of the influence of the hydrogen-ion concentration of the substrate on pectinase production. These two solutions were employed for two reasons: First, it has proved that *Rhizopus tritici* makes a good growth on both of them; second, they are easily prepared and thus available for experimental work at all times. The original solutions were prepared in sufficient quantity for a number of experiments. In the case of Czapek's nutrient solution the chemicals were put into a large enamel-ware vessel and the water added. The sugar was then added and the mixture heated for about one-half hour. A precipitate which formed during the process of heating was removed by filtering. The sweet-potato decoction was prepared according to the method described on page 862, except that 800 gm. of potato were used for each liter of water. From this point on the methods employed were the same for both solutions. Both solutions were made up to a strength such that when 30 cc. were diluted to 50 cc. they were of the desired concentration. The general method for adjusting a solution to a definite hydrogen-ion concentration described by Karrer and Webb (13) was employed. These authors, using Czapek's solution and beet decoction, worked out a method whereby the addition of a definite amount of acid, alkali, or water to 30 cc. of the stock preparation would give a definite hydrogen-ion concentration. They showed that it was possible to obtain a range of  $P_H$  1.2 to 10 + and 1.2 to 9.8 in beet decoction and Czapek's solution respectively by varying the amounts of N/5 HCl and N/5 KOH. The writers, selecting out of the series given by Karrer and Webb such  $P_H$  values as they wished to use, employed their method and made up Czapek's nutrient solution and sweet-potato decoction to these hydrogen-ion concentrations. Although the hydrogen-ion concentrations obtained were not identical with those obtained by Karrer and Webb, they gave a range sufficient to meet all the needs of these experiments. The final amount of the solution in each flask was 50 cc. Exactly 30 cc. of the stock solution (Czapek's solution or sweet-potato decoction) were added to each flask by means of a self-filling burette. The desired amount of water was added and the solutions were then autoclaved for 20 minutes at 15 pounds pressure. The required amounts of acid and alkali were then added to each flask under aseptic conditions. The burette was first sterilized with 95 per cent alcohol and then washed with sterile distilled water. Care was taken at every step to prevent contamination. The quantity of acid, alkali, or water to be added to make the solutions up to 50 cc. depended upon the hydrogen-ion concentration desired.

The acid and alkali with which the dilutions were made were held in two liter flasks. The flasks were plugged with rubber stoppers through

which two glass tubes passed, one of which was long enough to extend nearly to the bottom of the flask. The liquid was drawn out through the long tube. The other tube, through which the air entered the flask, was stoppered with cotton to prevent the entrance of contaminating organisms with the inflowing air. The flasks so prepared were autoclaved for 20 minutes at 15 pounds pressure.

The culture flasks, after being finally prepared, were allowed to stand for two days before inoculation in order to determine which if any of them were contaminated. In spite of all the possible precautions, some of the flasks in every experiment became contaminated. These were discarded and a portion of the remaining flasks were inoculated with a spore suspension of *Rhizopus tritici*. Some of the flasks were held as controls. The cultures were incubated in the dark at a constant temperature of 35° C.

The duration of the experiment varied from 5 to 8 days. At the close of the experiment a portion of the mycelium and of the solution on which the fungus grew was prepared according to methods already described and used for macerating tests. Another portion of the mycelium was used in obtaining the dry weight of the fungous material produced. A portion of the solution was used to determine the hydrogen-ion concentration and the amount of dextrose present in Czapek's solution only. The sugar was determined by means of a Fric saccharimeter. The hydrogen-ion concentration and sugar content of the controls and inoculated flasks were determined on the day the experiment was terminated.

Fifteen flasks of each hydrogen-ion concentration were prepared, 10 of which were inoculated. The remaining 5 were held as controls.

#### EXPERIMENTAL DATA

It has already been shown that pectinase was not produced in Czapek's nutrient solution when dextrose was used as a source of carbon. It was found, however, that when pectin was employed in the substrate as the only source of carbon in Czapek's solution a powerful middle lamellae dissolving enzyme was secreted. When pectin was combined with dextrose as a source of energy pectinase was produced, but its action was much slower, being presumably secreted in a much smaller amount. It was likewise pointed out that *Rhizopus tritici* produced a macerating substance on several vegetable decoctions (turnip, sweet potato, Irish potato, carrot, and bean) but not on synthetic media (Pfeffer's, Richard's, and Czapek's solution and beef bouillon). Furthermore, it has been demonstrated that some substance which causes maceration of raw sweet-potato disks is produced when *Rhizopus tritici* is grown on Czapek's nutrient solution. That this substance was an acid seems likely as some of the experiments to be detailed below will show. In view of the fact that Czapek's nutrient solution became rapidly acid when *R. tritici* was grown on it, it was suspected that the production of the acid interfered with the secretion of pectinase. It is also interesting to note in the way of comparison that when sweet-potato decoction in which pectinase was abundantly produced was used as a substrate the final acidity as measured in  $P_H$ , although increased, was never as high as in Czapek's solution.

A series of experiments were therefore outlined to determine what effect the original hydrogen-ion concentration of the substrate (Czapek's

solution, sweet-potato decoction) would have on the secretion of pectinase. The methods used in these experiments have been outlined already. The results of these experiments are shown in Tables III to VI.

Table III gives the results of experiment 1, in which the  $P_H$  values of the original control solutions (column 1) varied from 1.04 to 8.53. In this experiment the fungus grew for 5 days.

TABLE III.—(Experiment 1) showing hydrogen-ion concentration and dextrose content of the control and inoculated Czapek's solution, the time required by them to macerate sweet-potato disks, and the dry weight of the mycelium produced

$P_H$ of control.	$P_H$ of inoculated	Dextrose in control.	Dextrose in inoculated	Time required to cause maceration in control.	Time required to cause maceration in inoculated.	Dry weight of mycelium.
		Per cent.	Per cent.			Gm.
1.04	1.01	14.7	15.25	Complete in 5 hours	Complete in 5 hours	None
1.71	1.76	14.95	15.25	Complete in 24 hours	Complete in 24 hours	Do
2.62	2.27	14.95	14.55	Some in 48 hours	Some in 48 hours	0.0283
3.58	2.18	14.95	13.75	Nearly complete in 48 hours	Complete in 48 hours	0.0728
5.82	2.17	14.95	13.40	do.	do.	0.0972
6.01	2.18	14.95	13.30	Complete in 48 hours	do.	0.1005
6.57	2.15	14.95	13.00	do.	do.	0.1147
7.28	2.20	14.95	12.55	do.	do.	0.1357
7.61	2.32	14.90	12.20	Some in 48 hours	do.	0.1553
7.91	2.39	14.85	12.00	Very little in 48 hours	Nearly complete in 48 hours	0.1620
8.12	2.59	14.70	12.40	Doubtful if any in 48 hours	Very little in 48 hours	0.1748
8.53	2.66	14.55	12.40	None	Practically none in 48 hours	0.1780

In experiment 2 (Table IV) the fungus was allowed to grow for seven days. Carrot disks were macerated in about the same time as the sweet-potato disks. If there was any measurable difference the carrot disks were macerated in a little less time than the sweet-potato disks.

TABLE IV.—(Experiment 2) Showing the hydrogen-ion concentration and dextrose content of the control and inoculated solutions, the time required by them to macerate sweet-potato and carrot disks, and the dry weight of the mycelium produced

$P_H$ of control.	$P_H$ of inoculated.	Dextrose in control.	Dextrose in inoculated.	Time required to cause maceration in control.	Time required to cause maceration in inoculated.	Dry weight of mycelium.
		Per cent.	Per cent.			M.
1.67	1.63	15.9	15.9	Complete in 22 hours	Complete in 22 hours	None
2.67	2.22	15.9	14.9	Slight maceration in 30 hours	Slight maceration in 30 hours	0.0340
3.22	2.09	15.9	14.0	A little maceration in 30 hours	A little maceration in 30 hours	0.0766
4.39	2.18	15.9	14.0	do.	do.	0.0812
4.81	2.18	15.9	14.0	do.	do.	0.0814
5.69	2.12	15.9	13.5	do.	do.	0.1040
6.46	2.18	15.9	12.8	do.	do.	0.1283
6.78	2.46	15.8	12.2	No maceration in 30 hours	No maceration in 30 hours	0.1539
6.97	2.47	15.4	11.7	do.	do.	0.1679
7.23	2.59	15.4	11.1	do.	do.	0.1718
7.80	2.58	15.4	9.2	do.	do.	0.1734

TABLE V.—(Experiment 3) Showing the hydrogen-ion concentration of the control solution (sweet-potato decoction) at the beginning and end of the experiment, also of the inoculated solution, and the time required by the solution and mycelium to macerate sweet-potato disks

P <sub>H</sub> of control at end of experiment	P <sub>H</sub> of inoculated solution at end of experiment.	P <sub>H</sub> of control at beginning of experiment.	Time required to complete maceration.			
			In inoculated un-steamed solution.	In inoculated steamed solution	In uninoculated solution.	By hyphae ¼ gm. in 25 cc H <sub>2</sub> O.
			Hours.	Hours.	Hours.	Hours.
1. 27	1. 27	1. 28	2	2. 25	42	
2. 05	2. 10	2. 10	7-24	48	48	
2. 67	2. 55	2. 71	2. 25	24	Slight in 48	5.0
3. 80	3. 12	3. 83	2. 00	4. 5	None in 48	4.0
4. 66	3. 20	4. 69	2. 00	24. 0	do.	4.0
5. 06	3. 36	5. 20	2. 25	24	do.	5.0
6. 14	3. 48	6. 17	2. 25	48	do.	5.0
7. 12	3. 57	7. 19	2. 25	None in 48	do.	
7. 58	3. 60	7. 67	2. 50	do	do.	
8. 28	3. 69	8. 33	2. 50			5.5

TABLE VI.—(Experiment 4) Showing the hydrogen-ion concentration of the uninoculated sweet-potato decoction at the beginning and end of the experiment, also of the inoculated solution, dry weight of mycelium produced, and time required to macerate sweet-potato disks

P <sub>H</sub> of control at beginning of experiment.	P <sub>H</sub> of control at end of experiment	P <sub>H</sub> of inoculated solution at end of experiment.	Dry weight of mycelium.	Time required to complete maceration.			
				In inoculated un-steamed solution	In inoculated steamed solution	In uninoculated solution.	By hyphae ¼ gm. in 25 cc. H <sub>2</sub> O.
			Gm.	Hours.	Hours	Hours.	Hours.
1. 24	1. 24	1. 27		3. 00	6	18	
2. 01	2. 01	2. 02		24. 00	24	18	
2. 69	2. 63	2. 54	0. 0965	3. 25	48	None in 48	4. 75
3. 63	3. 57	3. 17	. 0115	2. 50	24	do.	3. 50
4. 33	4. 31	3. 29	. 1268	3. 00	24	do.	3. 75
5. 13	5. 05	3. 51	. 1214	3. 00	24	do.	4. 00
6. 05	6. 02	3. 51	. 1186	3. 00	24	do.	4. 50
7. 29	6. 32	3. 58	. 1388	3. 25	48	do.	4. 50
7. 99	7. 53	3. 72	. 1280	3. 25	None in 48	do.	4. 75
8. 30	8. 21	3. 86	. 1297	3. 50	do	do.	4. 75
9. 12	8. 86	8. 14	. 0693	None in 48	do	do.	5. 50

In experiment 4, the fungus felts from five flasks were collected and the dry weight determined. The average weight is given in Table VI. The mycelium from the other flasks was used in determining the rate of maceration.

An examination of Tables III to VI shows some interesting facts with respect, first, to the changes in hydrogen-ion concentration of the solution produced by the fungus; second, to the influence these changes have on the production of pectinase; third, to the consumption of sugar (in Czapek's solution); and fourth, to the amount of mycelium produced. It will be seen that growth was entirely inhibited at a P<sub>H</sub> of 1.7. The maximum limit of alkalinity for growth was not obtained, since the alkali in the solutions prepared to be about P<sub>H</sub> 11.0 appeared to react with the sugars, thereby reducing the alkalinity. It was found in general, however, that the growth was best in the most alkaline solutions so long

as it was not alkaline enough to inhibit growth. Growth was better in sweet-potato decoction than in Czapek's nutrient solution at approximately the same hydrogen-ion concentration.

The fungus tends in all cases to make the solution more acid. The degree of acidity reached was greater in Czapek's solution than in sweet-potato decoction. In experiment 1 (Table III) no growth took place at a  $P_H$  of 1.04 and 1.71 and the hydrogen-ion concentration was therefore not much changed. As the hydrogen-ion concentrations of the control solutions decrease, however, the change in the  $P_H$  value of the inoculated solutions becomes greater, as, for example,  $P_H$  8.53 (Table III) was changed to 2.66. It will be seen from Table III and the other tables also that the  $P_H$  of the inoculated solutions gradually increase with the decrease in the hydrogen-ion concentration of the control solutions.

That many fungi and bacteria make the substrate acid or alkaline is well known. Currie (4) showed that citric acid was produced by *Aspergillus niger* in a nutrient solution and Wehmer (20) gave the generic name Citromyces to a group of fungi which he believed was characterized by its ability to produce the same acid. Lafar (17), and Lind (18) found that oxalic acid was produced by certain species of *Penicillium*, *Botrytis*, and Citromyces. Weimer and Harter (21) showed that *Rhizopus tritici*, *Diplodia tubericola*, *Mucor racemosus*, *Penicillium sp.* and *Botrytis cinerea* increased the acidity of Czapek's nutrient solution when glucose was used as the source of carbon. Young and Bennett (25) found that *Fusarium oxysporum* when grown on Richard's solution with a  $P_H$  5 first made the solution acid and then alkaline so that in 40 days a  $P_H$  of 7.4 was reached. Bacteria appear to produce acid in the solution, though not to so marked a degree. For example, Jones (11) found that a strain of *Pneumococcus* when inoculated into a medium with an initial reaction of  $P_H$  7.0 grew poorly and developed a  $P_H$  of 6.2. If, on the other hand, the initial reaction was 7.6 a good growth and a final  $P_H$  of 5.4 resulted. *Streptococcus viridans* was found by Grace and Highberger (7) to change a broth with an initial hydrogen-ion concentration of between  $P_H$  7.1 and 7.3 to a  $P_H$  6.6 in 6 days. Similar changes in the hydrogen-ion concentrations were noted by Wolf and Harris (23) and by Karrer (12) with certain bacteria and fungi, respectively. A comparison of the results of other investigators with those obtained by the authors seems to indicate that *Rhizopus tritici* renders the substrate more acid than most fungi or bacteria. As a matter of fact, it will be seen that the degree of acidity produced ( $P_H$  2.09, Table IV) while not prohibitive of growth, which is practically stopped in a  $P_H$  1.7, closely approximates it.

From Tables III and IV it is seen that pectinase is not produced in Czapek's nutrient solution when adjusted to any of the hydrogen-ion concentrations tested. This conclusion is drawn from the fact that, although a certain amount of maceration took place in certain solutions, especially in those with the highest hydrogen-ion concentrations, there was just as much in the steamed as in the unsteamed solutions. In the solution with an initial  $P_H$  of 1.01 (Table III) no growth of the fungus took place and yet maceration was complete in 5 hours. When the initial  $P_H$  was 1.76, 24 hours were required to complete maceration. At this concentration no growth occurred. At all other concentrations there was a normal amount of mycelium produced and the final hydrogen-ion concentration of the inoculated solutions varied from  $P_H$  2.15 to 2.66



(Table III). At a concentration of  $P_H$  2.66 no maceration occurred. At the other concentrations about 48 hours were required for the cells to completely lose their coherence. There is therefore a degree of acidity such that the acid has no action on the middle lamellae in 48 hours ( $P_H$  2.66, Table III). At a  $P_H$  of 2.59 little maceration took place in 48 hours. In the remaining solutions (Table III) the acidity is about sufficient to dissolve the middle lamellae in 48 hours. At  $P_H$  1.76 maceration is complete in 24 hours and at 1.01 in 5 hours. Similar data are shown in Table IV.

The data presented in Tables V and VI were obtained by growing *R. tritici* on sweet-potato decoction adjusted to the different  $P_H$  values. These show that the decoction does not become as acid as Czapek's solution. No growth occurred in  $P_H$  1.27 and 2.03 (Table VI), but at all other concentrations about the same amount of mycelium was produced. The hydrogen-ion concentration of the solutions when there was growth was considerably increased; however, in no case was the increase as great as that found in Czapek's solution. At the two higher concentrations the maceration produced was doubtless due to the action of the acid, since in one case ( $P_H$  1.27, Table V), although there was no mycelium produced, coherence of the cells was lost in 2 hours, and in another case ( $P_H$  2.10, Table V) in 7 to 24 hours. In all the other concentrations the middle lamellae were completely destroyed in from 2 to 2½ hours in the unsteamed solutions. In the steamed solution there was practically no maceration in 48 hours.

The conclusions to be drawn from these data are, first, that pectinase is not produced in Czapek's nutrient solution at any hydrogen-ion concentration tried and, second, that its production in sweet-potato decoction is not interfered with when the hydrogen-ion concentration is not so high as to prevent growth. In other words, pectinase is produced by *R. tritici* when growing in sweet-potato decoction at any hydrogen-ion concentration that will permit its growth. Pectinase was also present in the mycelium.

The amount of sugar present in the control (not inoculated) and the inoculated solutions was determined for Czapek's solution only. At the higher hydrogen-ion concentrations no fungous growth took place and no sugar was used. As a matter of fact, there appears to be a slight increase in the amount of sugar present in the controls over that in the inoculated solutions, probably due to some concentration of the sugars as a result of evaporation. A measurable amount of sugar was consumed by the fungus at all other hydrogen-ion concentrations, the larger amount being at the higher  $P_H$  values. It will be noted that the amounts of sugar consumed at the lower hydrogen-ion concentrations are proportionately greater than in the less alkaline solutions. This is also true in the controls. No doubt this variation may be accounted for, at least in part, by the reaction between the sugar and alkali. The largest amount of mycelium was produced at the lower hydrogen-ion concentrations.

An examination of Tables III and IV shows that in Czapek's nutrient solution the amount of mycelium produced increases with the decrease in hydrogen-ion concentration, varying from 0.0283 gm. to 0.1780 gm. (Table III) and from 0.0340 to 0.1734 gm. (Table IV). No mycelium was produced at the highest hydrogen-ion concentration. In experiment 3, the dry weight of the mycelium was not determined, but in experiment 4 (Table VI) there was a more or less gradual increase in dry

material as the hydrogen-ion concentration decreased, with one exception. In the solution with an original  $P_H$  of 8.86, which is apparently too alkaline for normal growth, the dry weight was somewhat less than it was in the solution with a  $P_H$  of 8.21 (Table VI).

It has been intimated that the maceration which takes place in solutions after they have been steamed is due to the action of some one or more acids produced in the solution by the fungus. A number of acids have been shown by different investigators to be formed by fungi in nutrient solutions, among them being oxalic, acetic, and formic. These three organic acids, together with hydrochloric acid and sodium hydroxid, were made up to different hydrogen-ion concentrations and their ability to dissolve the middle lamellae determined. The results showed that maceration could be brought about by the acids in high hydrogen-ion concentrations and by the alkali at a  $P_H$  of about 12 or above. Oxalic acid at a  $P_H$  of 2.13 macerated raw sweet-potato disks in from 24 to 48 hours at a  $P_H$  of 2.44 in 72 hours. No maceration occurred in 72 hours by this acid at a  $P_H$  of 2.76. Acetic acid appeared to require more time to complete maceration than oxalic acid at the same concentration. Acetic acid of  $P_H$  2.13 macerated the disks in 72 hours, while at  $P_H$  2.40 the amount of maceration was very slight in that length of time and was entirely absent at a  $P_H$  of 2.44. In a  $P_H$  1.99 of formic acid maceration was nearly complete in 6 hours, at  $P_H$  2.16 in 24 hours, and at  $P_H$  2.23 in 54 hours. At hydrogen-ion concentrations of  $P_H$  2.40 and 2.57, there was slight and no maceration, respectively, in 54 hours by this acid. In full strength formic acid maceration was complete in 3 hours. Hydrochloric acid of a  $P_H$  2 will induce slow maceration. A comparison of these results with those obtained when Czapek's solution was used shows that the loss of coherence of the cells of sweet potato disks occurs in pure solutions of these acids at about the same hydrogen-ion concentration as in Czapek's solution and in about the same length of time. Oxalic acid macerates a little more rapidly than acetic acid at the same concentration, and formic acid a little more rapidly than either oxalic or acetic.

The results of these investigations seem to indicate that no pectinase is produced in Czapek's nutrient solution when glucose is used as a source of carbon. However, an acid is produced in sufficient hydrogen-ion concentration to cause a dissolution of the middle lamellae identical with that caused by pectinase. It was found also that the original  $P_H$  value of the solution had no influence on the secretion of pectinase. In all concentrations of Czapek's solution in which the fungus grew the hydrogen-ion concentration was increased, the greatest increase being in the solutions with the highest  $P_H$  values.

A middle lamellae splitting enzyme was produced when pectin alone was used as a source of carbon in Czapek's nutrient solution and to a less extent when combined with glucose. It had been suspected that the failure to obtain maceration in Czapek's solution was not due to the absence of the enzyme, but to its inactivation by the acid formed. Brown (2) showed that the addition of some of the organic and mineral acids to extracts of *Botrytis mycelium* retarded the action of the enzyme, the time required to macerate potato disks being increased with the increase in the concentration of the acid. A point was finally reached when inactivation was complete. The retarding action of all the acids, was found to be about the same up to a certain concentration beyond

which the retarding action of the mineral acids increased much more rapidly. A concentration was finally reached where maceration was caused by the acid. Brown in his studies used dilutions of normal acids, so his results can not be compared directly with those of the writers, who studied the action of the acids in terms of hydrogen-ion concentrations. That the acid formed in Czapek's solution did not inactivate the enzyme may be inferred from the following facts. When Czapek's solution, which was made acid ( $P_H$  1.8) by the growth of the fungus, was changed to a  $P_H$  of 7.8 and raw sweet-potato disks added no maceration took place. It had been previously shown that the enzyme was not inactivated at  $P_H$  of 10. The solution was not alkaline enough to inactivate the enzyme. Some of the data obtained with sweet-potato decoction may be cited as further evidence. It has been shown (Table V) that maceration in sweet-potato decoction is complete in  $2\frac{1}{4}$  hours at a  $P_H$  of 2.55. On the other hand, in Czapek's solution with a  $P_H$  of 2.66 (Table III) which is even less acid no maceration occurred in 48 hours. Although there is a difference in the composition of these two media, it is reasonable to assume that since the acid was not sufficiently strong in the sweet-potato decoction ( $P_H$  2.55) to inhibit the action of the enzyme it would not be able to do so in the less acid Czapek's solution ( $P_H$  2.66).

The investigations of the authors showed that the presence of pectinase in the mycelium grown on Czapek's nutrient solution could not be demonstrated, while it occurred regularly in the hyphae produced on sweet-potato decoction. If the enzyme was produced, its occurrence in the hyphae on Czapek's solution as well as on sweet-potato decoction would be expected. Furthermore, the enzyme in the mycelium, if it occurred there, would not be inactivated by the acid in the substrate, since the mycelium is always suspended in distilled water, which is practically neutral.

#### SUMMARY

(1) *Rhizopus tritici*, a fungus capable of causing the softrot of sweet potatoes was used in all these experiments. On suitable media it produces a powerful enzyme, called pectinase, which dissolved the middle lamellae of raw sweet-potato and carrot disks.

(2) A comparison of the production of the enzyme was made on the following media: String bean, prune, Irish potato, carrot, turnip, and sweet-potato decoction, and on Czapek's, Pfeffer's, and Richard's synthetic media and on beef bouillon.

(3) It was found that the cell wall dissolving enzyme was produced on all the vegetable media except prune decoction, but not on the synthetic media with glucose as a source of carbon or on beef bouillon.

(4) If pectin was used alone as a source of carbon in Czapek's nutrient solution, an active enzyme was produced. When pectin was combined with glucose the action on raw sweet-potato disks was feeble.

(5) When *Rhizopus tritici* was grown in Czapek's nutrient solution with glucose as a source of carbon the substrate became sufficiently acid to cause dissolution of the middle lamellae so that coherence of the cells was completely lost in from 6 to 24 hours.

(6) The production of the macerating principle by the fungus when growing on Czapek's nutrient solution and sweet-potato decoction was not influenced by adjusting these solutions to different  $P_H$  values. The enzyme was not produced in Czapek's solution at any hydrogen-ion

concentration tested. On the other hand, it was secreted in sweet-potato decoction at all concentrations tried.

(7) The best growth as measured by the dry weight of the mycelium took place at the lowest hydrogen-ion concentration.

(8) The amount of glucose consumed in Czapek's solution is correlated with the amount of mycelium produced.

(9) The substrate influences the production of pectinase; the hydrogen-ion concentration does not. The results indicate a well-marked qualitative action of the substrate on the production of the macerating enzyme, in that when vegetable decoctions which probably contain pectin are used and when nutrient solutions to which pectin is supplied as a source of carbon is employed pectinase is produced.

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# THE MICROSCOPIC ESTIMATION OF COLLOIDS IN SOIL SEPARATES<sup>1</sup>

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In a recent work upon soil colloids,<sup>2</sup> in which a description of the soil materials classified as colloids was given, it became desirable to compare the values obtained by an adsorption method for determining the colloidal content of soils with values obtained by some method independent of adsorption phenomena. The most satisfactory method for this purpose would be a mechanical separation of colloidal from noncolloidal soil materials. But it has been shown in previous publications<sup>2,3</sup> that a mechanical analysis probably does not effect a complete separation. However, it seemed possible that colloidal matter not separated from the soil by mechanical analysis might be estimated by microscopical means. Then the sum of the colloid extracted and that estimated microscopically would give a value for the total colloid in the soil by methods quite independent of adsorption.

For the purpose of separating the colloids from the coarser particles of the soils the samples were repeatedly treated by agitation with distilled water containing a trace of ammonia, rubbed with a rubber pestle, and the supernatant liquid decanted and centrifuged. From 40 to 60 such treatments yielded practically all of the colloid extractable by this method. For convenience of manipulation the soils were divided, during the washing and rubbing process, into the colloidal fraction made up of particles less than 0.001 mm. in diameter, a fine fraction made up of particles ranging approximately from 0.001 to 0.050 mm. in diameter, and a coarse fraction consisting of particles larger than about 0.050 mm.<sup>2</sup>

The adsorptive capacities of these fine and coarse residues indicated that they contained colloidal material.<sup>2</sup> Observations of the residues with the ultramicroscope showed that the mineral particles had been fairly well cleaned of adhering colloidal matter by the washing and rubbing, and that the colloidal material remaining in these fractions was in the form of lumps or aggregates made up, at least superficially, of very large numbers of particles less than 0.001 mm. diameter. But since the light coming to the eye from the ultramicroscope is reflected from the surfaces of the particles under examination it was possible that the colloidal aggregates thus observed were simply mineral grains completely coated with colloids or that, at least, the colloidal aggregates

<sup>1</sup> Accepted for publication Apr. 7, 1923.

<sup>2</sup> ANDERSON, M. S., FRY, W. H., GILB, P. L., MIDDLETON, H. E., and ROBINSON, W. O. ABSORPTION BY COLLOIDAL AND NONCOLLOIDAL SOIL CONSTITUENTS. U. S. Dept. Agr. Bul. 1122, 20 p. 1922. Literature cited, p. 19-20.

<sup>3</sup> DAVIS, R. O. E. THE INTERPRETATION OF MECHANICAL ANALYSES OF SOILS AS AFFECTED BY SOIL COLLOIDS. *Jour. Amer. Soc. Agron.*, v. 14, p. 293-298. 1922. Literature cited, p. 298.

inclosed minute mineral particles. The clean appearance of the obviously mineral particles was, of course, a strong indication that such was not the case, but the element of doubt remained nevertheless.

Under the petrographic microscope in transmitted light these colloidal aggregates were found to be almost universally transparent and, in the rare cases where this was not so, to be highly translucent. Therefore any mineral grain included within the colloidal aggregate would be readily visible provided that either its color or index of refraction was different from the color or index of the colloid. Observation of birefringence between crossed nicol prisms would, of course, readily differentiate between minerals and colloids regardless of similarity of color or refractive index. Such observation, however, showed that practically all of the colloidal aggregates were free from mineral inclusions. Presumably, aggregates containing such had been broken up by the repeated rubbing and washing process.

Therefore, since the residues consist of easily determinable minerals and colloidal aggregates, one readily distinguishable from the other, a microscopic estimation of the relative quantity of each in a given sample was easily obtainable. Owing to the extreme variation of size of particles a straight count would have been subject to large errors. Therefore, the estimation was made by means of a checker-work eyepiece micrometer, relative space occupied by the minerals and colloids being the basis of the calculation. It is believed, on the basis of results obtained with synthetic samples, that errors due to the varying thickness of the particles, both minerals and aggregated colloids, are fairly well balanced and do not appreciably affect the results. Quartz is the predominant mineral in the fractions, and since the specific gravity of this mineral (2.66) and that of the colloids (2.53-2.68) extracted from the soils are very near together, it was believed that differences in specific gravity would not give rise to any serious error.

In order to test this method of microscopic estimation, four synthetic samples were made up, each containing a known amount of colloidal material. Air dried Marshall soil colloid, which had been extracted mechanically and graded by means of the supercentrifuge, was mixed with quartz in the proportion of 1.2 gm. colloid to 0.8 gm. quartz and 0.8 gm. colloid to 1.2 gm. quartz. The first of these mixtures was ground dry to pass a 200-mesh sieve. In order to facilitate any tendency of the colloid to form coatings on the mineral particles, the second sample was ground wet, dried and subsequently rubbed lightly with a pestle to pass a 200-mesh sieve.

Since quartz is practically without cleavage, the particles formed by grinding have a tendency to assume shapes in which the dimensions vary little in different directions within the same particle, although the dimensions of different particles may vary widely. This statement also holds good for the colloidal aggregates. Such a similarity of fracture facilitates a microscopic estimation of the relative quantities of the different constituents present, and therefore does not afford a very difficult test of the method. But, since quartz is the predominant mineral constituent of soils, the results obtained with quartz are probably very near those obtained on the soil separates themselves.

For the purpose of testing the method under more unfavorable conditions, Orangeburg subsoil colloid, extracted mechanically and graded by the supercentrifuge, was mixed with hornblende which has a tendency

to cleave into oblong particles with the greater dimensions several times the magnitude of the smaller, thus presenting a widely different shape from the colloidal aggregates with which it was to be compared. Two samples were prepared consisting of 0.3 gm. colloid to 1.7 gm. hornblende and 0.4 gm. colloid to 1.6 gm. hornblende. The first of these was mixed dry and the second wet, as with the Marshall colloid-quartz samples.

The results of the microscopic examination of these samples are given in Table I.

TABLE I.—*Microscopic estimation of colloids in synthetic samples*

Sample.	Colloid present.	Colloid observed.
	<i>Per cent.</i>	<i>Per cent.</i>
Marshall colloid and quartz, mixed dry . . . . .	60	57
Marshall colloid and quartz, mixed wet . . . . .	40	45
Orangeburg colloid and hornblende, mixed dry . . . . .	15	12
Orangeburg colloid and hornblende, mixed wet . . . . .	20	23

These results showed that the colloid in the fine and coarse residues of the soils could be estimated with fair accuracy by this microscopic method.

Microscopic examinations were therefore made of the fine and coarse residues of eight soils which had been subjected to the repeated washing and rubbing process. The results are given in Table II. The quantity of the unextracted colloid in the fine and coarse residues, as determined by this microscopic method, are given in column 3. The additional data in Table II are given for the purpose of showing the relative amounts of "extractable" and "unextractable" colloids in the soils.

From Table II it will be seen that from 9.4 to 42.4 per cent of the soil was made up of colloids extractable by the methods employed. The microscopic examination of the residues shows that from 25 to 97 per cent of the fine residues and 2 to 25 per cent of the coarse residues were colloidal aggregates not extractable by the methods employed.

These results throw considerable doubt upon the results of several investigators who have determined the colloidal contents of soils and clays by purely mechanical methods. Hissink<sup>4</sup> and Sven Odén,<sup>5</sup> for example, have dispersed colloids in soils by very elaborate washing and rubbing processes. It is of course possible that they may have effected complete dispersion by their methods, but apparently no investigations were undertaken to ascertain whether, or to what extent, aggregates of undispersed colloids remained in their soil suspensions. Williams,<sup>6</sup> in the course of a very elaborate and painstaking separation of colloids from soils by washing, rubbing and boiling, did examine his residues microscopically. Details of his microscopic method are not given, but it is apparent that he used an ordinary chemical microscope and mounted his residues either in air or water. Owing to the wide differences in refractive indices between these mounting media and the ordinary soil minerals, scarcely more than surface phenomena could be

<sup>4</sup> HISSINK, D. J. DIE METHODE DER MECHANISCHEN BODENANALYSE. *In* Internat. Mitt. Bodenkunde, Bd. 11, p. 1-11. 1921.

<sup>5</sup> ODÉN, Sven. ÖBER DIE VORBEHANDLUNG DER BODENPROBEN ZUR MECHANISCHEN ANALYSE *In* Bul. Geological Inst., Univ. Upsala, v. 16, p. 125-134. 1918-19. Bibliographical footnotes

<sup>6</sup> WILLIAMS, W. R. UNTERSUCHUNG ÜBER DIE MECHANISCHE BODENANALYSE. *In* Forsch. Geb. Agr.-Phys. (Wolny), Bd. 18, p. 225-350. 1895. Bibliographical footnotes



observed under these conditions; and consequently the distinction between lumps of aggregated colloids and soil minerals would reduce itself purely to a question of personal judgment based on appearance. Colloidal aggregates might bear very close resemblances to corroded mineral particles and ferruginous or rutilated quartz, for example. Even with a completely equipped petrographic microscope careful cognizance must be taken of the pseudo-similarities of the optics as well as the resemblances of some crystalline and amorphous materials, such as the similarity between colloids subjected to strain and the matted structure of such minerals as chlorite.

TABLE II.—*Microscopic estimation of unextracted colloids in fine and coarse residues of soils*

Sample.	Quantity of fractions separated mechanically, as part of whole soil.	Quantity of unextracted colloid in residues determined microscopically, as part of residue.	Unextracted colloid in combined fine and coarse residues, as part of whole soil.	Unextracted colloid, as part of total colloidal material in soil.
	Per cent.	Per cent.	Per cent.	Per cent.
Cecil clay loam, soil:				
Colloid extracted.....	9.4			
Residue finer particles.....	7.6	74		
Residue coarser particles.....	83.8	4	9.0	48.9
Cecil clay loam, soil:				
Colloid extracted.....	9.4			
Residue finer particles.....	10.4	55		
Residue coarse particles.....	78.3	4	8.9	48.6
Huntington loam, soil:				
Colloid extracted.....	10.3			
Residue finer particles.....	21.9	48		
Residue coarse particles.....	64.0	8	15.6	60.2
Huntington loam, subsoil:				
Colloid extracted.....	13.3			
Residue finer particles.....	19.4	25		
Residue coarse particles.....	63.3	10	11.2	45.7
Sassafras silt loam, subsoil:				
Colloid extracted.....	14.4			
Residue finer particles.....	20.6	38		
Residue coarse particles.....	61.9	2	9.1	38.7
Sharkey clay, soil:				
Colloid extracted.....	42.4			
Residue fine and coarse particles combined.....	53.1	42	22.3	34.5
Sharkey clay, soil:				
Colloid extracted.....	31.5			
Residue finer particles.....	38.9	52		
Residue coarse particles.....	31.8	25	28.2	47.2
Vega Baja clay loam, soil:				
Colloid extracted.....	30.5			
Residue fine particles.....	38.6	97		
Residue coarse particles.....	31.8	14	41.9	57.9

The calculations given in column 4 of Table II show that from 8.9 to 41.9 per cent of the whole soil was composed of colloidal aggregates not extractable by the methods used. It should be mentioned, however, that the Vega Baja soil which showed a total colloidal content of 72.4.

per cent is an exceptional soil. Usually the quantity of colloid present is much lower. Basing the calculations on the colloidal matter alone, it is seen from the table that 34.5 to 60.2 per cent of the total colloids in the soils were not extracted by the methods used. Although this method of repeated rubbing and washing for the extraction of colloids from soils was carried out as carefully and thoroughly as possible, and although we are confident that no more than traces of colloidal material could have been extracted by further washing and rubbing, it by no means follows that other more efficient methods might not be devised.

As practiced in this bureau, microscopic estimation of the amount of colloidal aggregates in the fine and coarse residue is, at best, a long and tedious process. In order to insure fair sampling of the sample under study several mounts must be examined, and several areal counts should be made on each mount. Experience showed that a total of 10 to 12 counts on about four slides gave averages very comparable with those obtained by triple that number of counts on as many more mounts. Nevertheless when only 12 counts are made, the method is too long for routine application.

Unfortunately, the preliminary washing and rubbing are essential in order to remove coatings of the adhering extractable colloid. These coatings are rarely thick enough to interfere with the determination of the mineral; but the microscopic estimation of the quantity of colloid in the coatings would be extremely difficult and inexact. This necessity for washing the soils adds tremendously to the time consumed. Nevertheless, the method is of value in special studies.

#### SUMMARY

(1) A microscopic method for estimating the quantity of colloidal soil aggregates in soil separates is given.

(2) This method was applied to the residues left after extracting, by repeated rubbing and washing, all colloid possible from eight samples of soil. The results showed that from 34.5 to 60.2 per cent of the total colloidal material in the soil was in the form of colloidal aggregates not extractable by washing and rubbing.

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## MORPHOLOGY AND HOST RELATIONS OF *PUCCINIASTRUM AMERICANUM*<sup>1</sup>

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The orange rusts have always been considered as practically the only rusts of economic importance on species of *Rubus* in America. *Kuehneola uredinis*, it is true, is known to cause considerable damage to fruiting canes of certain cultivated varieties of blackberries. *Pucciniastrum americanum* (Farl.) Arth. is not rare on wild raspberries but its effects on the host have been found heretofore to be altogether negligible. Brief notes dealing mostly with its relationships to the European form *P. arcticum* have appeared from time to time but no reference has been made to damage to cultivated raspberries. An outbreak of this rust, therefore, of such severity as to cause defoliation and spotting of canes of certain varieties, becomes a subject of interest.

One of the native hosts of this rust is the wild *Rubus strigosus*, and like the orange-rusts and the stemrust, *Kuehneola*, the *Pucciniastrum* will no doubt be found wherever the natural hosts may grow. The rust has been reported rarely on *R. occidentalis*, but "*R. neglectus*," a hybrid between the red and the black raspberries, seems to be rather susceptible according to Farlow (9, p. 13).<sup>2</sup> At Bell, Md., where an outbreak occurred in 1922, Mr. G. M. Darrow has gathered together from all parts of the world a number of species and horticultural varieties of *Rubus* and has originated many new forms in connection with his breeding work. During July it was found that the leaves of several different hybrids were dropping off prematurely, leaving the canes entirely bare below. The lower surface of the leaves was evenly covered with uredospores, giving them nearly the same appearance as blackberry leaves heavily infected with the *Kuehneola*. The leaves at the growing tips were sometimes only slightly affected and the attacks seemed to increase in severity from the tips downward, showing that the rust had been present for some time.

Conical peridia (Pl. 1, C), each surmounted by a corona, were easily found on leaves just being attacked, and as the spores were identical with those found on the fallen leaves there can be no question that this defoliation was being caused by what has heretofore been considered a harmless rust. From July 28 to August 4 a survey was made to ascertain which species, varieties, and hybrids were being attacked. Mr. Darrow cooperated with the author in going over these records.

<sup>1</sup> Accepted for publication Jan. 22, 1923.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," pp. 893-894

Certain varieties were represented by as many as 75 plants, others by only 1 or 2, and conditions for infection were perhaps more favorable on one side of the field than on the other, so that a full report on these observations would not fully represent the actual degrees of susceptibility. Among others, the following seedlings were found to be quite badly infected: Ranere  $\times$  Cuthbert,<sup>3</sup> Cuthbert  $\times$  *R. lasiostylus*, La France  $\times$  Ranere, Van Fleet  $\times$  Ranere, Laxtonberry  $\times$  Ranere, Cuthbert, Gregg  $\times$  Cuthbert, Brighton  $\times$  La France, Brighton  $\times$  *R. lasiostylus*, *R. coreanus*  $\times$  Brighton, Shaffer seedlings, Shiebley  $\times$  King, *R. leucodermis*  $\times$  King, Newman 23  $\times$  *R. coreanus*, seedlings of Hailsham open pollinated, Syracuse open pollinated, Brighton selfed. The Ranere (St. Regis) was infected, but not as heavily as were the Cuthberts.

Certain hybrid seedlings were so severely infected as to result in almost complete defoliation, petioles and canes also being attacked. These very susceptible kinds include such hybrids as June  $\times$  Cuthbert, Latham  $\times$  Brighton, Latham  $\times$  Ranere, Erskine  $\times$  Ranere, *R. coreanus*  $\times$  Brighton, etc.

A number of varieties in this plot were only slightly infected, but, as previously noted, the number of plants in many cases was too small to prove that they were resistant. About 50 plants each of the following forms were carefully examined at this time, but no rust was found: *Rubus Idaeus*, open pollinated seedlings of the Mahdi (which is a cross between *R. Idaeus* and a European blackberry), *R. innominatus*  $\times$  Antwerp, *R. innominatus*  $\times$  King, *R. innominatus* (open pollinated seedlings). A few self-pollinated Cuthbert seedlings in this field showed no rust, but many plants of this variety in a garden near by were heavily infected.

Separated from these plots by about 40 rods was a third plot, in which several plants of wild *Rubus occidentalis*, *R. strigosus*, *R. leucodermis*, King, Cuthbert, Ranere, etc., were growing. A few pustules were found on one unnamed variety. A month later the rust was very abundant on the King, June, Newman 23, Cuthbert, and some was found on *R. strigosus*, but *R. occidentalis*, *R. Idaeus* (Hailsham variety), and 5 Asiatic raspberries were not affected.

Mention has been made of finding the rust on leaf stalks and canes. The cankers on the latter are sometimes 2 or 3 inches long. A species of *Gloeosporium* (not anthracnose) was also common in large cankers on the varieties whose canes were most heavily attacked by the rust. It is possible that this fungus became established in the lesions first formed by the rust; at any rate the combined effect of the two fungi will be to kill the canes. The calyx and carpels of late-fruiting or everbearing varieties were sometimes badly rusted. It is certainly curious to see the golden-yellow pustules deeply embedded in the juicy red carpels. There was more rust on the fruit of a late-flowering form of *R. strigosus* than on the leaves.

A press of work prevented a satisfactory examination of species of wild and cultivated raspberries in other localities to learn how common the rust was in the vicinity. It is clear, however, that the rust had found in these hybrids very susceptible hosts.

<sup>3</sup> The Black Pearl, Kansas, Cumberland, Gregg, and Plum Farmer, are American horticultural varieties of black raspberry which were originated as seedlings or by selection from *Rubus occidentalis*, of which *R. leucodermis* is a western variety; the Shaffer, Columbian, and Royal Purple are of American origin, probably derived from the form *Rubus neglectus*, which is a hybrid between *R. strigosus* and *R. occidentalis*; the Cuthbert, King, Victor, Erskine, June, Latham, Ranere, and Brighton, are American horticultural varieties of red raspberries, and related to *R. strigosus*; *R. Idaeus*, the Antwerp, La France, Laxtonberry, and the Hailsham, are European red raspberries; *R. coreanus* and *R. innominatus* are native of China.

An inspection of the berries grown at the Government farm at Arlington, Va., was not made until the latter part of September. From 6 to 50 hills each of Cuthbert, King, Ranere, Antwerp, wild *Rubus occidentalis*, Kansas, Black Pearl, Cumberland, Plum Farmer, Shaffer, Columbian, and Royal Purple are grown. The Cuthbert red raspberry was the most severely attacked; much defoliation had resulted, and the remaining lower leaves were well covered with uredinia. Considerable rust was found on the Columbian, Royal Purple, and the Victor. The Ranere (St. Regis) and King varieties bore some rust, but not enough to cause any damage. No rust was found on any of the black raspberries grown here, and as *R. occidentalis* (at Bell, Md.) showed no rust it may be assumed that our black raspberries are very resistant. It is not strange to find that crosses between the black and the red varieties in many cases prove to be very susceptible. Whether the strain of the rust which made its appearance in the vicinity of Washington, D. C., is more virulent than that heretofore found on *Rubus* "*neglectus*" and *R. strigosus* is not known, but it is evident that many of the hybrids at Bell are more susceptible than either of the parents.

The host is occasionally erroneously identified by collectors, especially in the case of the red raspberry, which is often confused with the black raspberry. For example, in the Path. Coll., B. P. I., is found No. 2280, N. A. Uredinales, one gathering at Madison, Wis., October 6, 1906, and another at Mackinac, Mich., September 6, 1894, in which the host is given as *R. occidentalis* when it is a red raspberry. Arthur (2, p. 190) states that *R. occidentalis* was erroneously given as a host for *Pucciniastrum americanum* in Bull. Torrey Club (1, p. 468). In the same article a specimen from Algoma, Wis., was referred to *P. arcticum*, and the host is given as *R. occidentalis*. The rust is clearly of the *P. americanum* type, and the host is *R. strigosus*. Davis (6) has shown that *R. occidentalis* can be infected by *P. americanum* from *R. strigosus*. We have not found pustules of this rust on the wild black raspberry in the vicinity of Washington, D. C., but more careful search would very likely result successfully. The rust is undoubtedly more widely distributed than is commonly supposed. Specimens on cultivated raspberries have been referred to other species by mistake. In the Path. Coll., B. P. I., we find one packet of the rust on a red raspberry originally labeled *Lecythea Ruborum* Lev.; No. 32, Economic Fungi, Seymour and Earle, *Phragmidium Rubi-Idaei* (P) Wint. is *Pucciniastrum americanum*. One gathering of *Pucciniastrum* on *R. strigosus* was sent to the author as orange-rust in response to a request for red raspberry plants infected with the *Gymnoconia*.

#### THE MORPHOLOGY OF THE UREDINIUM

Farlow (9) recognized that the rust which he had found on a hybrid raspberry differed somewhat with respect to the shape of the peridium from *Pucciniastrum arcticum* of Europe. The peridium was more sharply conical than that of *P. arcticum*, which is said to be low and broad. He called attention to a good illustration of the peridium of the American form by Dietel in Hedwigia (7, p. 331) under the name *Phragmidium gracile*. Farlow proposed the variety name *americanum*, which Arthur (1, p. 468) later raised to specific rank. Davis (5) has shown that *Pucciniastrum arcticum* is very common in Wisconsin on *Rubus triflorus* and questioned at that time whether the differences in the morphology noted by Farlow may not after all be due to host differences. He is now convinced (6),

however, that the two forms are distinct and reports the work of one of his students who failed to infect *R. strigosus* with uredospores from *R. triflorus*, whereas *R. strigosus* and *R. occidentalis* were infected with spores from *R. strigosus*. The reader is not informed just what was done in the way of attempting to infect *R. triflorus* with spores from either host. The leaves of *R. arcticus* and *R. triflorus*, practically devoid of tomentum on the lower surface, are certainly quite different in texture from those of our common raspberries. No one would question that there may be, as we have shown, certain hosts that are more susceptible than others, and it is possible that differences in texture of tissues attacked by the rust could very well account for the differences or modifications in the form and size of sori which originally led Farlow to suggest that the form on *R. strigosus*, etc., be called *P. arcticum* var. *americanum*. The presence of *P. americanum* at Bell, Md., where pustules occurred so abundantly on leaf blades, midribs, petioles, canes, floral envelopes, and even on the fruit, offered a rare opportunity to study the morphology of the sorus.

Our sections show that there is a great variation in the form of the uredinia developing on the same plant. Some are sharply conical with coronate peridia; others are low and broad. Pustules were found to vary in size from  $50\ \mu$  to 1 mm. in diameter. The peridia of uredinia developing on the under side of the leaf (Pl. 1, C) conform to the type described by Farlow. On the larger veins and especially on the petioles they tend to grow much larger and round out, sometimes becoming nearly globular (Pl. 1, B). The corona is not conspicuous in such peridia, especially where they are rather deep-seated, originating beneath three or four layers of cells.

Sori on canes are much flattened, elliptical or lenticular (Pl. 1, D, E, F, G, and 3, A). In such cases the heavily cutinized epidermis effectively prevents the protrusion of the peridium (Pl. 1, G), so that the peristomal cells and most of the peridium often disappear through disorganization. Spores are discharged through the passageway opened by the crushing and thrusting aside of the peridial and host cells above (Pl. 1, A).

Deep-seated sori are apt to occur in the calyx and fruit where the apex of the peridium seldom appears through the epidermis. Adjacent sori merge by the dissolution of side walls, large saclike cavities which are packed with spores resulting. Sori that do not lie much over two or three cells deep in the tissues beneath the epidermis are erect (Pl. 1, F), broadly elliptical, or lenticular. While individual sori on canes are large, they increase in length by coalescence (Pl. 1, G). Very frequently the vegetative hyphae mass together, crushing aside the host cells of the cortex. If these primordia lie near the forming cork layers, or lie deep down in the cortex, the uredinia will be inverted, the "peridium" being formed on the inner side (toward the center of the cane) (Pl. 1, D, E).

Two sori may originate from the same primordium, one having a peridium on the side toward the epidermis, the other on the side next to the cork cambium, which lies quite deeply embedded in the cortex. The sori shown in Plate 1, F, originate from separate primordia; the one above is erect, the other inverted.

#### ORIGIN OF THE SORUS AND ITS PERIDIUM.

The origin of the sorus is most easily studied from sections of the rust as it occurs on leaf stalks. The vegetative hyphae are not well provided with a granular or stainable cytoplasm, but the cells giving rise to the

primordium increase in size and their contents become granular and stainable and the nuclei show very distinctly (Pl. 2, A). Cells become arranged side by side in a compact plectenchyma. The terminal cells which eventually constitute the peridium elongate rapidly, lose their granular cytoplasm and nuclei by disorganization, and act as buffer cells to push aside and crush the host cells above. In Plate 2, B, two or three terminal cells at each end of the section are more heavily shaded in the drawing to indicate that they were more deeply stained than the others; the final divisions by which peridial cells arise will soon take place. In this way the sorus continues to increase in breadth for some time. In the formation of the teleutospores of species of *Gymnosporangium* (8) terminal cells of a sorus primordium function purely as buffer cells and entirely disappear as the sorus matures. It is the subterminal cells from which the teleutospore buds are formed. This is not the case in *Pucciniastrum americanum* on petioles, where the buffer peridium persists more or less and is usually recognizable in an old sorus, while the subterminal cells disappear.

Later the author will consider spore formation as found in *P. agri-moniae*, the species studied by Ludwig and Rees, but if Plate 2, A and B, of this paper is compared with their figure 1 in Plate VIII it will be seen that the only essential difference is in the layer of somewhat shorter or more flattened subterminal cells which the author found in various stages of disorganization, indicated by the way these cells take the orange G. In their figure the subterminal cells in the four central rows are not as thick as those below them. Sections presenting such features as are shown in Plate 5, A, of this paper certainly can be interpreted to mean that the layer of cells, b, which extends across the sorus, is composed of active basal cells, since these cells take the gentian violet stain rather deeply, while the cells of the "hyphal plate" and vegetative cells below take scarcely any stain. The cells s take the orange and safranin and certainly appear to be degenerating. The cells u will develop into spores. Toward the center of this sorus space has been formed and the intercalary cell below the spore is elongating into a stock as it disorganizes. If this method of spore formation were continued, the uredospores would be borne in chains, and essentially there is very little difference between the two methods. In very compact deep-seated primordia, conditions may be such as to necessitate the formation of the first spores in this way. A greater upward thrust against the overlying tissue would result if the basal cell elongates as a whole or at its upper end in preparation for division. In Plate 5, B, are shown the first spores formed in a sorus where the pressure was soon relieved at the center by a break in the overlying tissues. Large wedge-shaped uredospores with perfectly definite stalks can be seen. Nearer the margin of this sorus there was still considerable resistance at the left and above, so that the spore initial buds developing from the basal cells, b, are pushing out to the right where the tissue was less compact. The relationship of peridial cells, p, intercalary cells, i, and basal cells, b, is very evident in sections of this sorus. Further evidence that pedicellate spores occur in more mature sori is scarcely necessary. At the right in Plate 5, C, is shown a perfectly typical pedicellate spore. At the left three adjacent basal cells are budding; nuclear division is occurring in the cell at the middle.

If the tangled mass of hyphae constituting the sorus primordium is exceptionally large the development of a clear-cut peridium is more or



less interfered with, and uredospores often appear to be formed from almost any cell of the vertically oriented hyphae. The dark area in the sorus shown in Plate 1, G, indicates where disorganization of abnormal spores or of a portion of the primordium tissue is occurring, so that space will be provided and the host tissue above ruptured. More detailed studies of the process involved in spore production in such atypical sori should be made in order to determine to what extent these apparent variations from true form are really the results of abnormal conditions.

The "hyphal plate" layer mentioned by Ludwig and Rees is commonly present along the base of the sorus in *P. americanum* and seems to be almost continuous with the peridium, as shown in Plate 1, B. The origin of this tissue is perfectly clear. The columns or chains of cells constituting the plectenchymatous primordium are the much enlarged, vertically oriented branches of the ordinary hyphae which originally formed a small tangled mass at the commencement of the uredinium. These hyphae can not be distinguished from the purely vegetative hyphae by the size, form, or staining properties of their cells. Now, the sorus increases in breadth simply by the lateral extension of branches from this undifferentiated tissue. Certain branches grow out vertically and parallel the other columns of cells and form peridial, intercalary, and basal cells in regular order, so that there are bound to be platelike layers of cells, especially along the base at the margins, which connect up with the peridium.

In order to secure further proof of the homology of intercalary cells and uredospore stalks, the author gathered and fixed material showing various stages in the development of uredinia of *P. agrimoniae* and *P. hydrangeae* on November 2 at Occoquan, Va. A study of sections of *P. agrimoniae* showed that Ludwig and Rees have given a good figure of a young sorus. There is ample evidence, on the other hand, proving that intercalary cells which degenerate are cut off below the peridium (Pl. 5, D, E) and that such cells are also formed with at least the first uredospores (Pl. 5, F). Many cases were observed where there could be no doubt that the basal cell forms one or more buds, spore initials (Pl. 5, G), each of which in turn divides to form a spore supported by a stalk. The stalk may disappear without much or any elongation. A few other stages in spore formation are figured here showing that *P. agrimoniae* does not differ materially in the organization of its uredinium from *P. americanum*.

Much of the material of *P. hydrangeae* was probably gathered too late in the season, as most of the sori sectioned had aborted or undergone degeneration after having developed the chains of cells constituting the primordium. Such stages are, in this condition (Pl. 5, J), apt to be misleading. In a few cases the uredinia appeared to be healthy and normal. Some evidence was found showing that the intercalary cells are cut off as the peridium is formed (Pl. 5, K). Certain irregularities in the structure of this tissue need further study. One could find stages where the first spores were being formed, showing that the uredospores originate as a result of the cutting off of a spore initial from the basal cell as a whole and not as a bud. In this species the cell supporting the spore must be considered the homolog of a true stalk cell or pedicel. Young spores supported by intercalary cells are shown in Plate 5, H.

Plate 3, A, B, shows more clearly that the peridium is composed of the terminal cells of the sorus primordium. The subterminals take the

orange stain more deeply than the others, therefore this layer is easily recognized in sections. Disorganization, followed by collapse, occurs, so that these interstitial cells are represented finally by more lines of degeneration products.

#### METHODS OF SPORE FORMATION

The formation of uredospores in *Puccinia* from a definite layer of basal cells which send out buds (spore initials) is well understood. Nuclear division is followed by cell division, cutting off from the spore initial the stalk cell below and the spore above. Such spores are thus not borne in chains, even though the basal cells may continue to bud and give rise to a number of spores.

Liro (10, p. 490, 492, 493) states that the uredospores of *Melampsorella cerastii* and *M. feurichii* are borne in caeomalike sori, that is, in chains. Magnus (12), while confirming Liro regarding the first species, disagrees with him as to the second, figuring stalked spores in a sorus of *M. feurichii*, and further holds that it would be impossible for a spore to be formed out of such stalk cells. He creates the new genus *Milesina*, especially characterized by stalked uredospores, leaving in *Melampsorella* *M. cerastii*, which he and Liro agree has catenulate spores.

Ludwig and Rees (11), who made a study of the uredinium in *P. agrimoniae*, find that the apical cells of the primordium elongate and their contents stain less deeply. The terminal cells become the first peridial cells, and the other cells of the chains are the uredospores, no intercalary cells being formed. These authors further state that the spore chains arise from a layer of basal cells just above a "hyphal plate layer," which is a tissue extending across the base of the sorus and connecting at the margins with the peridium. Stages in spore formation are not shown, although they follow Magnus in their explanation of the probable method. Since intercalary cells were not found, they suggest that the peridium is formed in a way analogous to that in the ordinary aecidium. They also suggest that the genera now included in the *Pucciniastratae* may be separated into two subgroups, the fern rusts having pedicellate spores in one group, and *Pucciniastrum*, *Melampsorella*, *Melampsoridium*, characterized by spores borne in chains with each chain maturing but one spore at a time, in the other.

The writer has not made an extensive study of the order of cell division in spore formation in *P. americanum*, but there can be no question that the uredospores are frequently borne singly on pedicels. On the other hand, if the very important fact that intercalary cells are present is disregarded, there is considerable evidence showing that the first spores, especially in deep-seated sori on petioles and canes, are borne somewhat as Magnus describes for *Melampsorella*. In other words, in *P. americanum* the first spore initials arise as the result of tangential divisions of basal cells and not by budding. Any claim that the upper daughter cell may sometimes become a spore at once, the sister cell remaining as a basal cell, would be difficult to disprove.

#### FORMATION OF THE OSTIOLE

The formation of the ostiole and the peristomal cells in *P. americanum* can be followed very readily by studying sections of sori which are not very deeply seated. About the time the first spores appear in the cavity beneath the central part of the buffer peridium, it will be seen that a few

cells at the center or apex are beginning to disorganize and collapse at their lower ends (Pl. 1, A, and 5, B). As the peridium is lifted these central cells sustain considerable pressure from the sides so that they eventually become distorted, compressed below, enlarged above, and are finally thrust somewhat out of place as they break through the epidermis. The spinelike thickenings which are being formed during the rupturing processes may in some way facilitate the final dislodgment of the peristomal cells in older sori. The cell ps in Plate 4, E, is slightly more than half the length of that of the adjacent peridial cell, p. Originally these two cells were the same length; therefore, the difference seen later is due, not to the thickening of the wall of the lower end, as thought by Farrow, but to disorganization which is not confined to the peristomal cells alone. It is interesting to see that while the lower end of such cells is becoming disorganized and distorted the cytoplasm and nuclei in the upper part are still active, holding the gentian violet stain to the last or until the spines are formed.

It is now a well-recognized fact that peridial cells of an aecium are the homologs of aeciospores, being merely the specialized terminal cells in the chain of spores. The figures in this paper suggest that these homologies can be carried over to the uredinium of *Pucciniastrum*, where the peridial cell and intercalary cell below are comparable to the uredospore and its stalk, which supports the theory first advanced by Sappin-Trouffy (13, p. 86) and further defended by Christman (4).

Stalked spores arising from budded basal cells were not found in the limited study of *P. hydrangeae* made by the author. Such types as are shown in Plate 5, I, suggest that in older sori the intercalary cell may elongate to take the form of a stalk and that the basal cell may bud the same as we find in the other species.

It is a certainty that spores are sometimes formed atypically in *P. americanum*, so the author has hesitated to interpret the figures in Plate 3, which show (at the right) spores without stalks or supporting intercalary cells. The author has relied on the positive evidence offered by finding intercalary cells and pedicellate spores in just such sori, rather than on such negative evidence as these figures might be interpreted to afford. In the orderly process of spore formation in uredinia there is a hymenial layer of basal cells from which spore initials regularly arise, but all sorts of abnormalities are liable to occur here as in other fungi, so that a spore might be formed from almost any cell, peridial, intercalary, or basal. Every well-nourished cell in the primordium is potentially sporogenous, but usually one of the daughter cells of the spore initial is in effect sacrificed, thus providing additional food for the one that is to become the spore; the other, although incidentally becoming a disjuncting intercalary cell or an elongated stalk, finally degenerates.<sup>4</sup>

Magnus' figure 7, Plate XIV (12), in support of his view is not convincing. He may have overlooked the stalk cells or their homologs, intercalary cells, as he did the peridial cells in *Hyalospora* which Bartholomew (3) shows very plainly to be present in this genus. Ludwig

<sup>4</sup> J. Kursanov, in a recent paper which has just come to hand (*Recherches morphologiques et cytologiques sur les Uredinées*. Bul. Soc. Nat. Moscow, v. 31, p. 1-129, pl. 1-4, 25 fig. Oct., 1922), traces the development of terminal sterile cells in the uredo sori of *Puccinia allii*, *Triphragmium ulmariae*, *Uredo (Pucciniastrum) pirolae*, and *Hyalospora polypodii-dryopteridis*. He figures intercalary cells directly beneath the sterile terminal cells in the last two species and states that they arise by the division of the subterminal cells. This may very well be their method of origin in the species of *Pucciniastrum* which the writer has under discussion, but it would not affect their homology. Instances of the cutting off of the intercalary cell from the upper part of the aeciospore initial are well known. Kursanov has not followed fully the development of the uredo spores in *Pucciniastrum pirolae*, so that the reader is left in doubt as to whether they are provided with true stalk cells or not.

and Rees, as noted, state that the uredospores of *Pucciniastrum agri-moniae* are borne in chains and that the terminal cells of the chains elongate to become the peridial cells, the other cells developing one by one into uredospores as described by Magnus for *Melampsorella*. These authors did not find intercalary cells either in the spore chains or below the peridial cells. If this were true, the peridium would be formed in a manner analogous to that of the aecidium, as they pointed out.

Some of the strongest hints as to the trend in evolution are suggested by what it is thought are atypical processes; but if in addition to the method of spore formation which the author has observed in *Pucciniastrum* it should be rarely found that every cell below the peridium in the cell chains develops into a uredospore, as claimed by Ludwig and Rees, the doctrine of homologies would be weakened. The buffer cell in the telium of *Gymnosporangium* may be the homolog of the peridial cell in *Pucciniastrum*; this depends on just how the first intercalary cell in the latter is cut off. It is not the homolog of the peridial cell of the ordinary aecidium, but rather it is comparable to the hymenial basal cell or its sister peridial initial. If the uredospores of *Melampsorella cerastii* are always borne in chains, and those of *Hyalopsora* are borne singly on pedicels, then the species of *Pucciniastrum* form a good series of connecting links, *P. hydrangeae* being more nearly like *Melampsorella*, especially if we should find intercalary cells in the latter.

The complete life history of *P. americanum* is unknown. Three other species of the genus have their aecial stage on *Tsuga* and two on *Abies*. The occurrence of our rust on canes of the current season suggests that the fungus may live through the winter, thus obviating the necessity for the alternate aecial stage in certain localities. No explanation has been offered to account for the presence of the deep-seated, inverted sori on canes, which are very common. It is possible that the inversion may facilitate spore discharge the following spring as the cortical parenchyma peels off, exposing the cork tissue below. Such inverted sori were not found on leaves or petioles.

It has been shown that the form and size of sori vary greatly, depending upon what tissue they are found, indicating that *P. arcticum* and *P. americanum* are not distinct species. In the event that it can be shown that *Rubus triflorus* and *R. arcticus* can not be infected with spores from *R. strigosus* and related forms, distinct names for the strains or biologic forms might still be desirable.

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## PLATE 1<sup>a</sup>

*Pucciniastrum americanum* (Farl.) Arth. on June X Cuthbert Red Raspberry

A.—Section through the center of a young uredinium on leaf stalk. Four layers of host cells lie above the sorus. Cells next to the sorus have been crushed by the expansion of the buffer tissue. Disorganization of cells at the center. Uredospores formed irregularly, intercalary cell replaces stalk.

B.—Vertical section through a mature sorus on a leaf stalk. Only two layers of host cells above. The buffer tissue has been flattened by the pressure from within and will persist more or less as a peridium, although the cells at the center are fully collapsed and will soon disappear. No corona would have been formed. Uredospores stalked.

C.—Vertical section of sorus on lower side of leaf. One cell of the corona showing.

D.—Inverted broadly elliptical sorus on young cane. "Peridium" or buffer tissue facing the cork cambium, which is deep-seated; e, epidermis; ck, cork layers.

E.—Inverted sorus still more deeply seated. Buffer tissue directly against the cork.

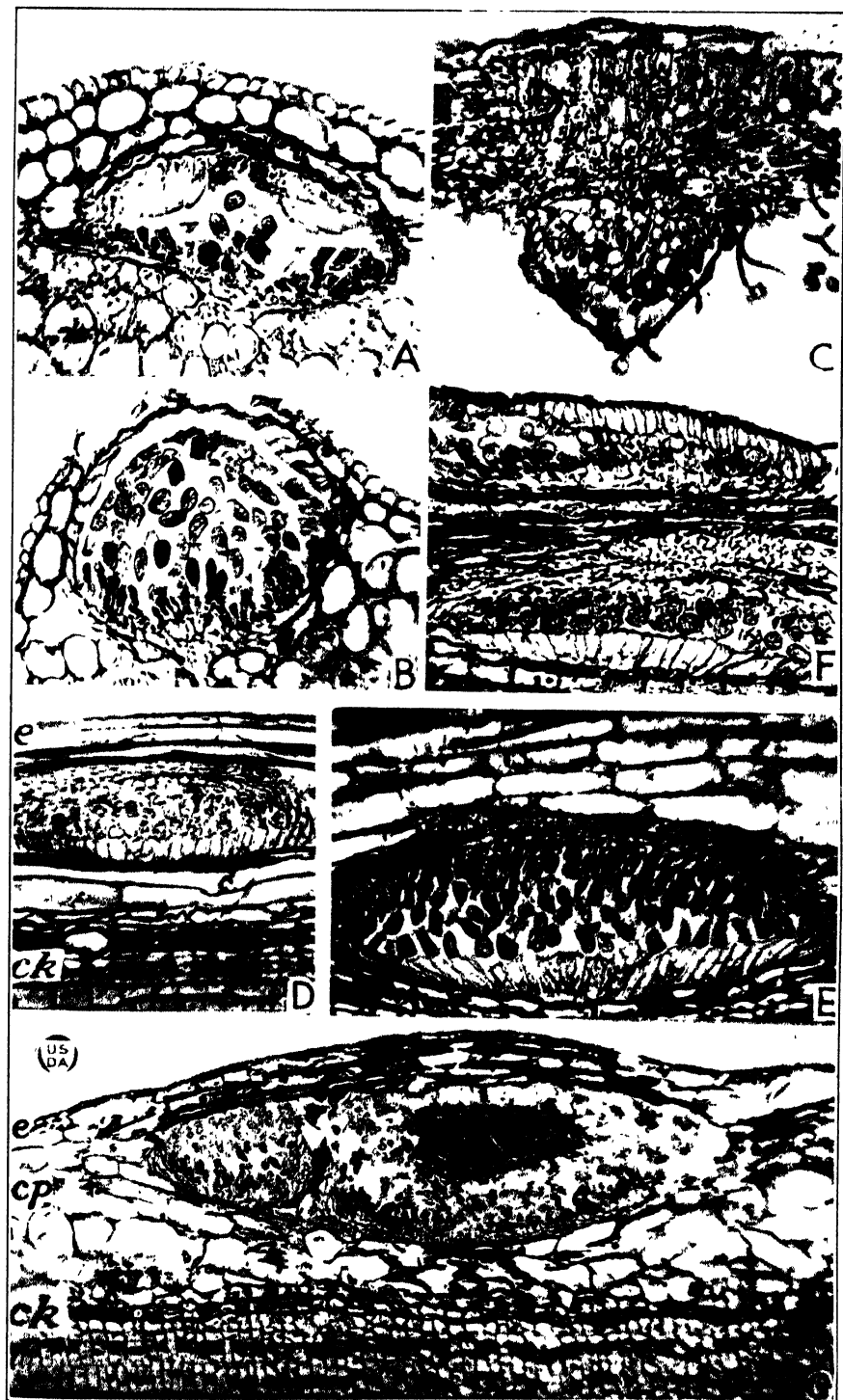
F.—Two lenticular sori on a cane. The upper one lies just beneath the epidermis and is erect; the lower is inverted.

G.—Erect double sorus on large cane. Peridium of the part at the left has entirely disappeared, cavity filled with spores. At the right peridium still evident although disorganizing. Degeneration of tissue at the center of the sorus (dark area), normal spore production below. Note the position of the epidermis above the sorus and of the cork cambium below it; e, epidermis, cp, cortical parenchyma, ck, cork.

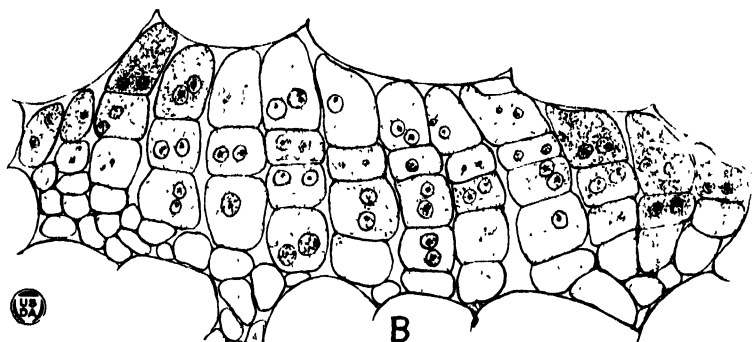
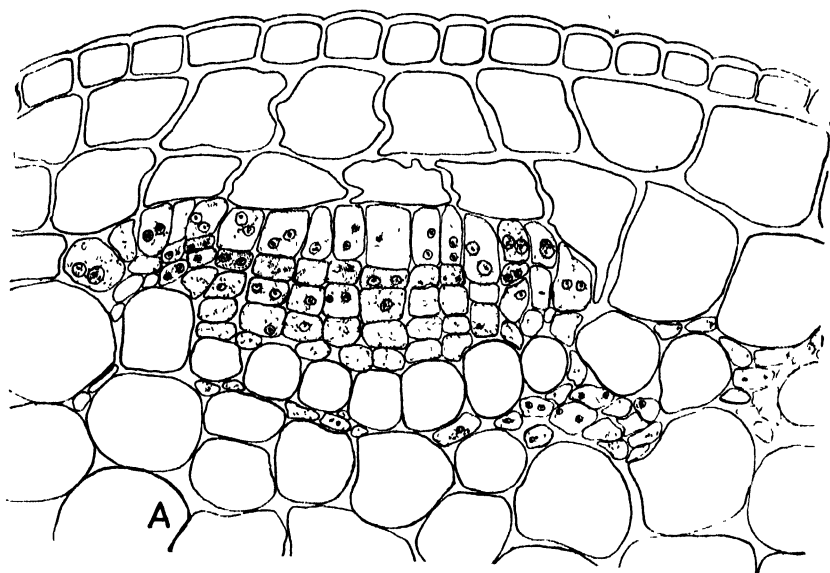
B to F, photographed with Leitz 8 mm. lens and No. 10 oc.; A, 4 mm., 6 oc.; G, 16 mm., 10 oc.

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<sup>a</sup>The photographs shown in Pl. 1, and all the sections which were studied in the preparation of this paper were made by Miss Ruth Colvin.







## PLATE 2

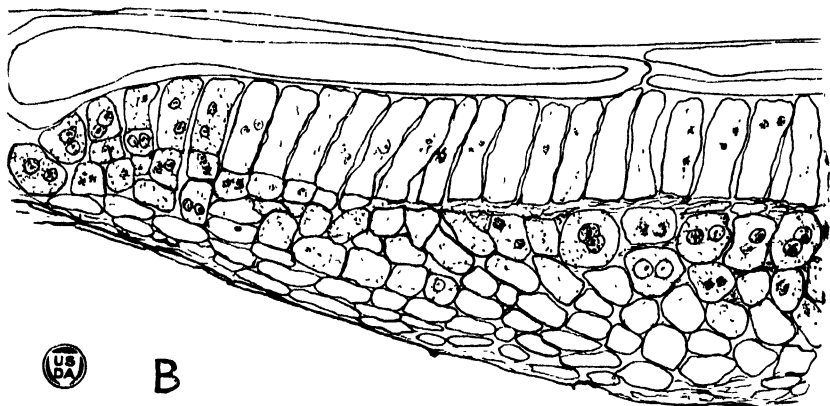
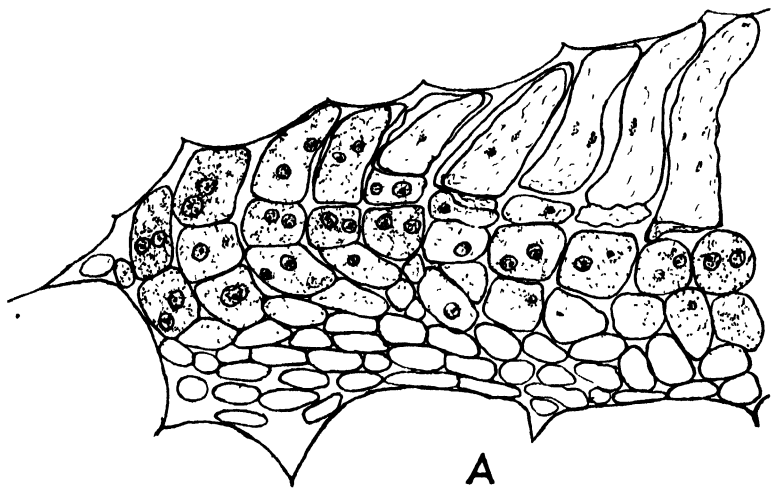
A.—Primordium of sorus on leaf stalk of raspberry, showing its position in the host tissues. Peridial or buffer cells, each accompanied by a disorganizing intercalary cell, at the center of the sorus. Cell division by which intercalary cells are formed has not occurred at the margins.

B.—Primordium of sorus on petiole of raspberry. Terminal cells elongating, intercalary cells disorganizing. Cells at the margin just before division.

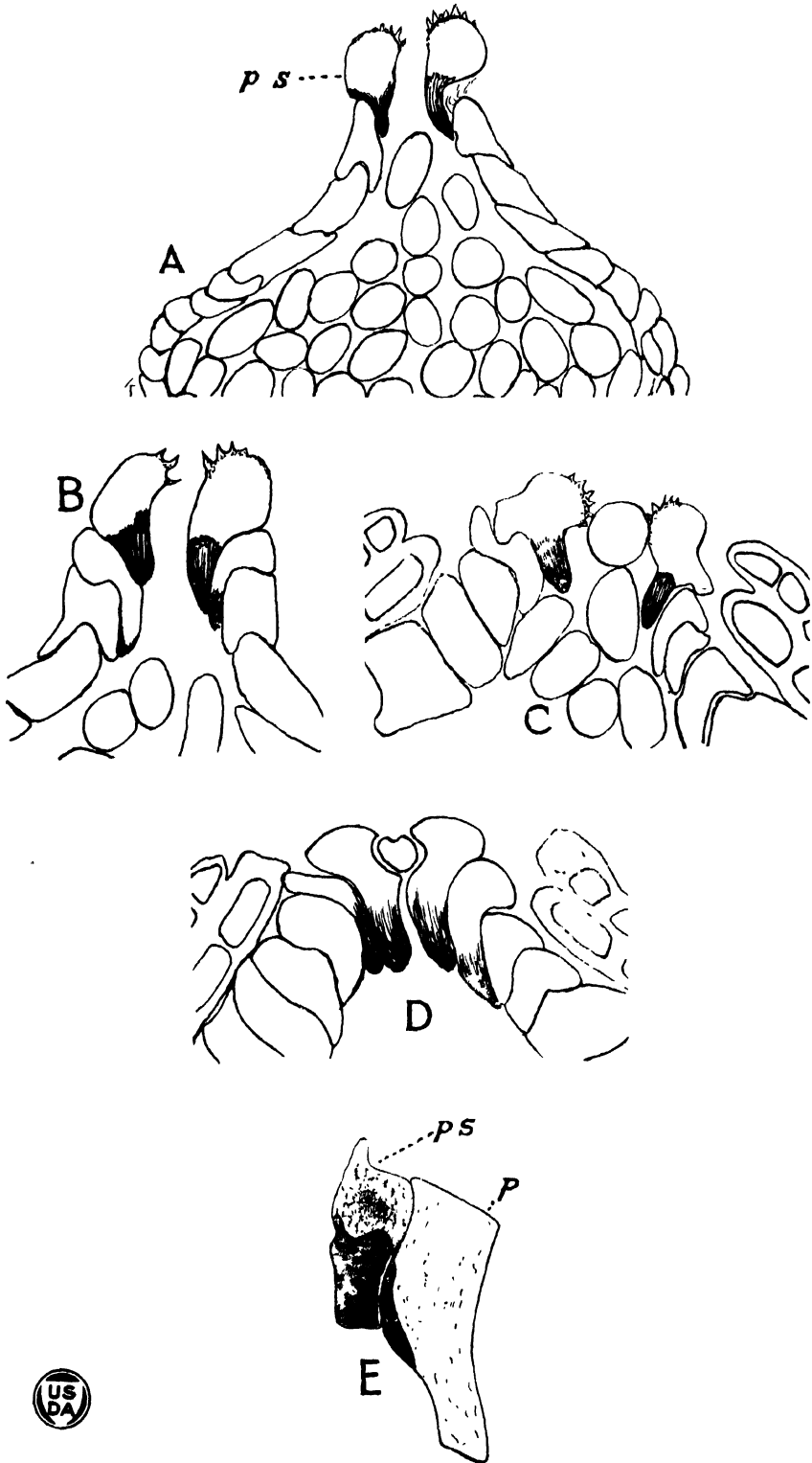
### PLATE 3

A.—Part of section of a young sorus on leaf stalk. Peridial cells fully elongated at the right, contents disappearing, intercalary cells collapsing.

B.—Part of section of a small and rather flat sorus on a cane. Buffer tissue of erect cells; intercalary cells in all stages of disorganization. Five uredospores at right center.



B



#### PLATE 4

The origin of peristomal cells in *P. americanum*. Deep shading of the peristomal cells, PS, indicates disorganization; P, peridial cell. (See text for further explanation.)

## PLATE 5

### *P. americanum*

A.—Columns of cells in a young, compact sorus. Uredospores arise by division of spore initial cells: p, peridial cell; i, intercalary cell; u, uredospore; s, stalk; b, basal cell.

B.—Formation of stalked spores in a sorus in which space was provided by the early rupture of overlying tissues. Spore initial buds are arising from basal cells (at the left) so that the first spores will be borne on stalks.

C.—Uredospores formed from budding basal cells. Nuclear division occurring in the bud from the second basal cell.

### *P. agrimoniae*

D.—Row of cells at margin of sorus, wedge-shaped intercalary cell below the peridial cell p.

E.—Fully elongated peridial cells supported by intercalary cells beneath which are normal basal cells, not spores.

F.—Typical spore initial bud si, from basal cell (right); cell chain consisting of young uredospore, intercalary cell, basal cell, and "hyphal plate" cell; stalked spores.

G.—Short stalk cells. Whether we find two spores or only one connected with the basal cell may depend on the way the cell is sectioned.

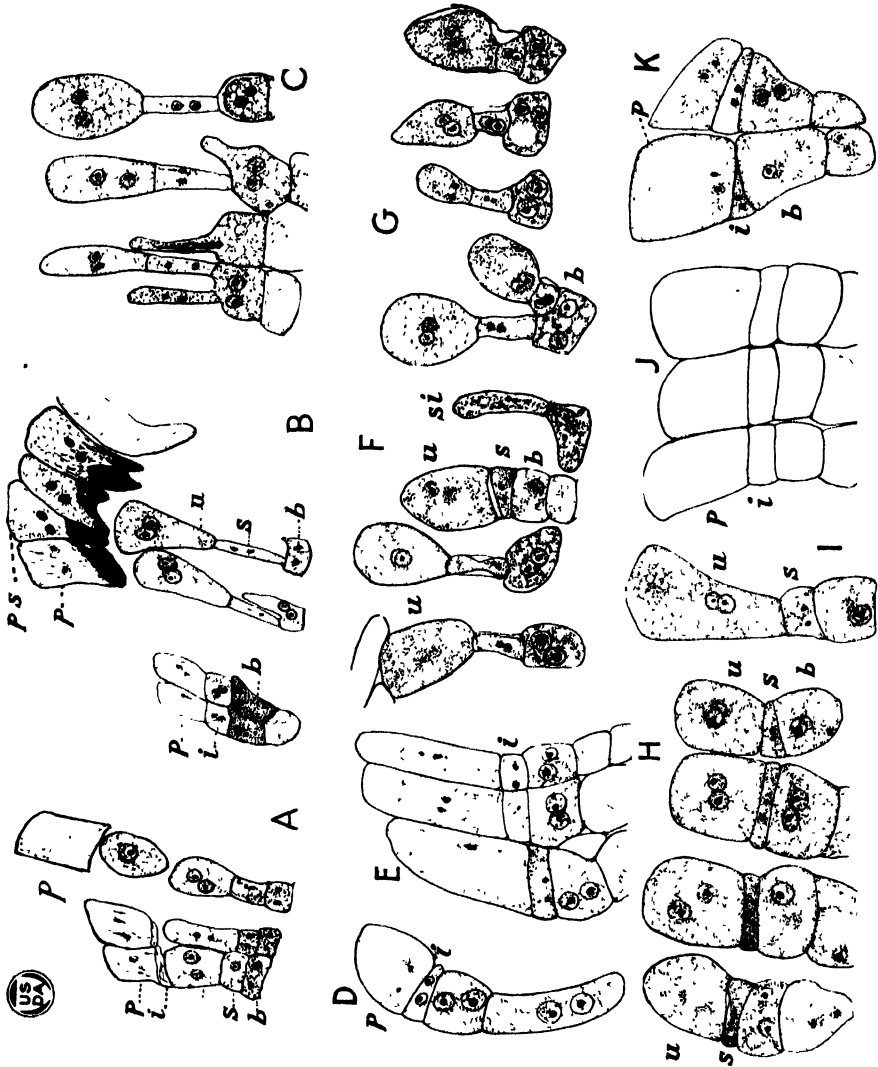
### *P. hydrangeae*

H.—Four young uredospores, u, each supported by an intercalary cell.

I.—Intercalary cell elongating somewhat.

J.—Chains of cells in an aborted sorus. Intercalary cells beneath the peridium.

K.—Cell chains at the margin of a normal young sorus; wedge-shaped intercalary cells degenerating.







# WATERY-ROT OF TOMATO FRUITS<sup>1</sup>

## A PHYSIOLOGICAL FORM OF OOSPORA LACTIS; EFFECT ON THE HOST; PENETRATION OF THE CELL WALLS BY ENZYMIC ACTION

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### INTRODUCTION

A new rot of tomato fruits closely resembling the rot caused by *Bacillus carotovorus* has frequently been found in shipments of southern grown tomatoes and sent to the Office of Cotton, Truck, and Forage Crop Disease Investigations by the Bureau of Markets' inspectors since the spring of 1921. It is also prevalent in the vicinity of Arlington, Va., and Washington, D. C.

This rot is characterized by the extremely watery appearance and condition of the affected tissues, by the absence of any other discoloration, and by the occasional oozing of water from the surface—features by which it can readily be distinguished from other fungous softrots. The rotted areas usually develop in the form of sectors extending from the stem scar toward the blossom end. In very humid air they are partly covered with a white velvety to granular fungous growth, but under average atmospheric conditions this is absent. The illustration shown in Plate 4, A, is fairly typical of this rot except for the low point of origin and the presence of a surface growth.

That this rot has frequently been mistaken for the rot caused by *Bacillus carotovorus* is quite likely, as the two are similar in macroscopic appearance. However, it differs from the bacterial rot in rapidity of development; in fact, fruits kept in the laboratory 10 days after this rot had made considerable progress were not completely softened, while those infected by *B. carotovorus* usually collapsed in about 3 days.

As an examination of the affected fruits obtained from the Bureau of Markets always disclosed the presence of an *Oospora*, experiments were made to determine the relation of this fungus to the disease.

### INVESTIGATION

#### MATERIAL AND METHODS

The *Oospora* used in the inoculations was grown chiefly on carrot agar, as it grew better on it than on most other kinds of media. It grew well also on turgid raw carrots kept in a moist atmosphere, producing a distinct rot (Pl. 4, C), but this material was used only for morphological comparisons.

In the inoculation work, green, ripe, and partly ripe tomato fruits free from blemishes were submerged for 30 minutes in a 1:1000 aqueous solution of bichlorid of mercury, washed in distilled water, and inoculated with a pure culture of the *Oospora* obtained from the rotted fruits.

<sup>1</sup> Accepted for publication Jan. 22, 1923.

This treatment caused some discoloration of the fruits but no softening. It had no visible effect on susceptibility to infection by this fungus, for fruits treated with weak solutions of bichlorid of mercury or formaldehyde as well as fruits not treated were quite as readily infected.

Controls were liberally used in all the experiments. Both the controls and the inoculated fruits were usually kept in closed glass chambers to prevent contamination from the air, but inoculated fruits kept in open dishes were quite as readily infected.

The decomposition of the cell constituents was observed on roots of carrots and on green tomato fruits. Sections of carrot roots, chiefly from the heart, and of green tomato fruits, 250 and 500  $\mu$  thick, respectively, and free-hand sections of varying thickness were used as fresh material. Pieces of green tomato fruits from spots 2 days old were treated with Flemming's medium killing and fixing solution, embedded in paraffin, and sectioned and stained for the study of fixed material.

The cultures were made as described by Brown (3)<sup>2</sup> on thin layers of media (carrot decoction, and beef bouillon + 2½ per cent glucose) in small flasks or Petri dishes, inoculated with an optimal quantity of spores, and kept at a temperature of 22° to 26° C.

The action of the enzymes on the host cells was determined by means of the live organism, the ground-dried organism, the extract from the ground-dried organism, the filtrate from cultures, and the alcoholic precipitate from the filtrate.

The organism was separated from the culture medium by passing the liquid through a double layer of Whatman's No. 50 filter paper. A few small cells passed through the filter but showed no signs of germination during the experiment.

The enzymic material was precipitated by adding 4 volumes of 95 per cent alcohol to each volume of the filtrate. The liquid was then filtered and the precipitate washed in 95 per cent alcohol, dried in a warm air current, dissolved in a very small quantity of distilled water, and used immediately.

The ground-dried organism was used in aqueous suspension. The extract of the same material was prepared by soaking the powdered fungus in water for 24 hours.

The decomposition experiments were run for 24 hours at temperatures ranging from 24° to 45° C. Growth of microorganisms was prevented by adding chloroform or toluene to the liquid in the proportion of 10 to 25 per cent, but duplicate experiments in which no antiseptic was used were also made. Chloroform and toluene inhibited growth without apparently hindering the action of the enzym.

#### RESULTS OF INOCULATION

The results obtained with both pricked and unpricked fruits are summarized in Table I.

Of the 277 pricked fruits inoculated, 208, or 75 per cent, became infected. The ripe fruits seemed to be somewhat more susceptible than the green fruits, but both were easily infected through punctures. The infections of unpricked fruits took place only through the stem scar. This was frequently observed in preliminary experiments not recorded. Moreover, it appears to be the cause of the position of the rotted areas of this type on most shipped fruits.

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 905.

TABLE I.—Results of inoculating tomato fruits with the *Oospora* associated with watery-rot

	Green.	Rip- ening.	Ripe.	Total.	Fruits inoculated.				Fruits infected.			
					Green.		Ripening.		Ripe.		Total.	
					Num- ber.	Per cent.	Num- ber.	Per cent.	Num- ber.	Per cent.	Num- ber.	Per cent.
Pricked . . . . .	160	90	27	277	118	74	64	71	26	96	208	75
Unpricked . . . . .	16	7	5	28	0	0	0	0	2	0	0	0

\* Infected through the stem scar.

Although these infections were obtained with an apparently pure culture of *Oospora*, the possibility of contamination by *Bacillus carotovorus* was not overlooked. The cultures were repeatedly plated and examined microscopically for the presence of bacteria but no evidence of bacterial contamination was found in either the plates or microscopic mounts. Moreover, the fungus pricked into halved potato tubers caused only a slight superficial growth, even when allowed to stand for a considerable period of time, while *B. carotovorus* produced a very decided rot (Pl. 4, B). The infections of the inoculated tomato fruits were therefore unquestionably caused by the fungus.

The stems and leaves of 15 vigorous tomato seedlings about 4 inches tall were thoroughly sprayed with the *Oospora* spores and kept in a moist chamber 60 to 72 hours but no infections developed. Ten similar tomato seedlings were thoroughly pricked in stems and leaves and smeared with the spores of *Oospora*, but the fungus was unable to invade the tissues. It therefore seems to have little if any parasitic action on tomato plants.

#### MORPHOLOGY OF THE FUNGUS

The *Oospora* causing watery rot of tomato fruits consists of a hyaline, septate mycelium with granular contents and numerous short branches arising near the septa at an angle of about 45° from the main filament (Pl. 3, A) and other longer branches of a two-or-three-forked type (Pl. 3, B-D).

The hyphae vary from 2.5 to 7.5  $\mu$  in diameter. The younger branches are narrower than the main filament but the taper is very gradual.

Reproduction is accomplished by the breaking up of the hyphae into their cells which serve as spores. The branches shown in Plate 3, A, divide into numerous short cells which round at the ends (Pl. 3, E, F, H) and separate. The rounding appears to begin in the apical cells (Pl. 3, G), but occurs in all cells of a branch almost simultaneously. At a certain stage of their development these cells appear to be chains of spores arising from the main part of the mycelium (Pl. 3, E, F), but by the time they lose their coherence, or shortly afterward, the main filament itself breaks up by cell division and separation into numerous cells of different lengths, which round at the ends, and when short are often indistinguishable from those formed from the lateral branches. They are hyaline and granular and are capable of germinating immediately.

The transverse diameter of these sporelike cells (oidia), which for convenience will be referred to hereafter as spores, varies from 2.5 to 7.5  $\mu$ ; the length from 3.2 to 40  $\mu$  and in some cases even to more than 60  $\mu$ .

Germination of the spores may start from the end (Pl. 3, I, L), from the side (Pl. 3, J, K), or from a corner (Pl. 3, I, M). Development from a corner of the cell is quite similar in point of origin to the development of the branches shown in Plate 3, A.

#### COMPARISON WITH *OOSPORA LACTIS*

The *Oospora* obtained from rotted tomato fruits shipped from the Gulf States was compared morphologically with the *Oospora lactis* that commonly grows on the surface of tissues in the cracks of ripe tomatoes; with two cultures of *O. lactis* received from Dr. Charles Thom, one isolated by him from pickle scum, the other sent to him from Germany; and with an *Oospora* isolated from green tomato fruits affected by watery-rot at the Government Experimental Farm, Arlington, Va. The two *Oosporas* causing watery rot and the one from Germany did not always break up into their cells so readily and completely as those from the cracks of ripe tomatoes and from pickle scum, but this habit varied with the age of the culture and with the kind of culture medium used. Aside from this difference, which was not constant, and slight differences in quantity of mycelial growth, there were no peculiarities, except in parasitism, by which one form could be distinguished from the others. The *Oospora* received from the Gulf States and the one obtained from green tomato fruits at the Government Experimental Farm reproduced the watery-rot in a large percentage of the fruits inoculated, but the others appeared to have no such parasitic action on tomato fruits. It would seem, therefore, that the *Oospora* causing watery-rot of tomato fruits, both in the Gulf States and at the Government Experimental Farm, is a physiological form of *O. lactis*. Consequently we have given it the trinomial *O. lactis parasitica*, form phys., to distinguish it from the parent species. As it is indistinguishable morphologically from *O. lactis*, it needs no further description.

It would be interesting to know how this form compares with the 9 varieties of *Oospora lactis* that Schnell (8) grew on sliced potato tubers, but as he made no inoculations on tomato fruits an accurate comparison of their parasitism is impossible. Six of the 9 varieties grown on sliced potatoes, 4 of which grew also on sliced cucumbers and 2 on plums, produced a discoloration of the potato tissues which would distinguish them from the form isolated from green tomatoes. Two of the others produced slimy colonies on sliced potatoes—a character not obtained with the watery-rot fungus. The remaining strain, viz, Oid. l. 557, made a feeble growth on potato tubers, agreeing in this respect with the one isolated from tomato fruits affected by watery-rot, but this has no significance with reference to its parasitism on tomato fruits.

#### TEMPERATURE RELATIONS

The effect of temperature on growth and infection by *Oospora lactis parasitica* is shown in Table II.

The minimum temperature obtained for germination of the spores, for growth of the mycelium, and for infection of pricked tomato fruits was approximately 2° C., the optimum temperature 30°, and the maximum 38.5°, except for infection of fruits, which was 37.5°. As there was a difference of 1° to 2° between the temperatures of adjoining chambers and some fluctuation within each chamber, these temperatures are only approximately correct.

TABLE II.—Relation of temperature to growth and infection by *Oospora lactis parasitica*

Temperature.	Growth on culture media. <sup>a</sup>	Infection of pricked tomato fruits.	Germination of spores.
	°C.	°C.	°C.
Maximum.....	38.5	37.5	38.5
Optimum.....	30.0	30.0	30.0
Minimum <sup>b</sup> .....	2.0	2.0	2.0

<sup>a</sup> Carrot agar and glucose agar.<sup>b</sup> The experiments on the minimum temperature were run about a month.

## EFFECT ON THE HOST

## ACTION ON THE CUTICLE

It was shown in the inoculation experiments described above that this fungus is unable to infect uninjured tomato fruits except through the stem scar or other similar areas not covered by the cuticle. This is also illustrated in Plate 1, A. The fungus lay in masses on the surface of the fruit but was unable to penetrate it. However, when once inside the fruit it invaded the epidermal cells quite readily.

## ACTION ON THE PROTOPLASM

Invasion of the host cells is soon followed by a gradual consumption of their protoplasmic contents. Some of the steps in this process are illustrated in Plate 1, in which B and C show an early stage, D a medium early stage, and E-J late stages. This action of the fungus on the protoplasm of the invaded cells is quite evident soon after the penetration of the wall, as it causes dissolution of the protoplasm in advance of the growing tip (Pl. 3, R). These transparent areas, or digestion vacuoles immediately surrounding the filaments, are shown more fully in Plate 1, B-D, and Plate 3, U. It would seem from these figures that the proteolytic enzyme secreted by the fungus acts chiefly in the region of the growing tip. There was apparently no preference for the nucleus, as it often remained intact after most of the other cell contents had disappeared.

Quite different results were obtained with the ground dried organism, the filtrate, and the alcoholic precipitate from the filtrate. Repeated experiments with these substances produced no visible effects on the protoplasm. The failure to obtain a proteolytic action with any of these substances may be due to a rapid deterioration of the enzyme, to inhibitors, or to lack of suitable technic.

## ACTION ON THE MIDDLE LAMELLA

The growing fungus, as shown in Plate 1, dissolves the middle lamella slowly, causing the loss of cell coherence. This loss of coherence and the breaking up of the fungus filaments into their individual cells produce the extremely watery consistency which distinguishes this rot from other softrots in which the fungus filaments remain intact and hold the host cells together. A similar action was produced on the middle lamella by the ground dried organism, the extract from the ground dried organism, the filtrate, and the alcoholic precipitate from the filtrate of cultures 2 to 3 days old, but this activity diminished as the age of the culture

increased. In fact, the alcoholic precipitate from cultures 6 days old and the filtrate from cultures 21 days old produced no visible effect on the middle lamella. This deterioration or inhibition of the action of the pectinase from age is quite different from the rapid action obtained with pectinase from cultures of *Bacillus carotovorus* 21 days old.

#### ACTION ON THE CELLULOSE

The passage of this fungus through the cell walls is shown by the drawings in Plate 3, N-T, and by the intracellular filaments in the photomicrographs of Plate 1. The drawings in Plate 3, N-T, were made from partly destroyed cells of a disintegrating area of the fruit. Only the tips of growing hyphae are shown passing through the somewhat wavy and more or less separated walls. The penetration of the cell walls of normal cells by young germ tubes was also observed by means of the microscope. The more important details of this process will be described later.

No visible effect was made on the walls by the filtrate, by the alcoholic precipitate from the filtrate, nor by the mycelium in the presence of sufficient chloroform or toluene to inhibit its growth. Moreover, bits of filter paper placed in fresh cultures of the fungus and allowed to remain there for 10 days to 2 weeks did not disintegrate. The fungus filaments passed between the fibers, causing the paper to tear apart somewhat more readily after the breaking up of the hyphae than similar bits of paper kept in distilled water, but microscopic examination of the fibers failed to reveal any corroding effects. There was apparently no chemical action on the filter paper.

It might seem from the foregoing facts that pressure rather than enzymic action enables the fungus to penetrate the cell walls as described for *Pythium debaryanum* by Hawkins and Harvey (7), but further observations do not substantiate this means of penetration for *Oospora lactis*.

Before a fungous filament can penetrate a cell wall by means of pressure, it must attach itself to the wall, or, if in a cell, to the protoplasm in order to prevent pushing itself away from the wall as it elongates. Spores of this fungus germinated either in water or in culture solution do not attach themselves to the slide or the cover slip. Moreover, when germinated in cells of tomato fruit tissue they do not adhere to the wall or the protoplasm. When the tip of such a sporeling comes into contact with the wall, its more or less continuous growth in length usually pushes it aside, which causes it to slide along the wall. Not infrequently the position of the whole filament is thus changed, as well as the position of other sporelings lying in contact with it. In fact, such a filament may even shift its position in such a way as to remove its tip some little distance from the wall. Such short filaments go through the walls more easily at the corners of the cell because there is less chance to slide along the wall. When a filament has passed through a wall it pierces other walls more rapidly because the anchorage thus obtained holds the growing tip against a single point better than does the free spore end of a germ tube that has no anchorage.

The phenomena accompanying the penetration of a cell wall by a germ tube of this fungus throw some light on the means by which it is accomplished. By placing spores of the fungus on the top of thin sections of tomato fruit tissue mounted and covered on a glass slide and furnished with a constant supply of water, the growth activities of the

spores that settle in the cells as well as the penetration of the wall and the effects resulting from it are easily observed by means of the microscope. Some short germ tubes lying near a wall and approaching it perpendicularly go directly through it without the use of any support or anchorage to increase their pressure. The opening made in the first half of the wall is a hole, not a basin or general depression such as would be produced by pressures, although the second half of the wall, i. e., the wall of the cell undergoing invasion, is sometimes pushed back. Whenever an invading filament completely fills the hole in the first half of the wall it attains a certain amount of anchorage which no doubt enables it to make some use of its growth pressure. This causes the second half of the wall to bend back before the filament has passed through it (Pl. 3, P, T), but it is not essential to the penetration. Moreover, this bending is usually absent, because the hole made in the first half of the wall, as shown by focusing sharply with the microscope, is usually a little larger than the filament (Pl. 3, V). It is also destitute of radiating cracks or fragments such as would be likely to accompany the bursting of the wall by pressure. By pressing the cover slip with a needle so as to produce vertical and lateral movements the angle between a germ tube and the wall through which it has passed may be varied more than  $90^\circ$ . This is caused by the pressure of the liquid against the filament, which changes its position in the wall without bending it at the edge of the hole. The two ends of such a filament usually move in opposite directions, especially when the part extending through the wall is three or four times as long as the part in the original cell. If the filament were rigidly fixed in the wall, the angle between it and the wall would not change unless the filament were bent at the edge of the hole. Moreover, an occasional filament can be made to slide in the hole. These phenomena are possible only when the hole is larger than the filament. In view of these facts, it would seem that this fungus invades the cells by means of a cellulose-dissolving enzyme (cellulase) secreted by the growing tips while in contact with the wall.

#### COMPARISON WITH *BACILLUS CAROTOVORUS*

As this *Oospora* and *Bacillus carotovorus*<sup>3</sup> cause similar rots of tomato fruits, a comparison of their effects on the host is interesting. *Oospora* invades the cells and destroys the protoplasm before it causes much separation of the walls (Pl. 1, B-F). Although it is also found in the intercellular spaces, it apparently makes little use of them except as passages. *Bacillus carotovorus*, on the other hand, remains in the intercellular spaces until it destroys the middle lamellae of the adjoining cell walls (Pl. 2, A-D) and enters the cells usually after it has destroyed their coherence. That an earlier entrance is sometimes effected, however, is evident from Plate 2, E. In later stages (Pl. 2, F-H) it not infrequently fills the cells.

#### ENZYM VERSUS PRESSURE

If we assume that the penetration of the cuticle by all fungous parasites is by "sheer mechanical pressure," as concluded for *Botrytis cinerea* by Brown (4, 5) and by Blackman and Welsford (1) and for *Sclerotinia*

<sup>3</sup> An excellent history of the work done on the softrots of vegetables (also tomato) caused by the *Bacillus carotovorus* group of organisms is published in "An Introduction to Bacterial Diseases of Plants," by Erwin F. Smith (9).



*Libertiana* by Boyle (2), we may be greatly misled. This would apply also to the conclusions of Hawkins and Harvey (7) regarding the penetration of cellulose walls. Fungi produce a variety of enzymes by means of which they decompose substances and obtain food. Moreover, it is quite likely that they produce many more enzymes than have been isolated. Failure to isolate an enzyme therefore does not disprove its existence, especially as the physiological factors involved in the production of enzymes and the activators and inhibitors controlling their activities are little understood. Moreover, the observation by Hasselbring (6) that the cavities made in the waxy covering of *Berberis Thunbergii* berries by the anthracnose fungi are much larger than the germ tubes and the conclusions by Ward (10, 11) from an exhaustive study of the brownrust of bromes that there is absolutely no relation between thickness of walls, number and size of stomates, hairs, and other mechanical structures and immunity to rust, present a striking contrast to the pressure theory of parasitic invasion. That growth pressure often accompanies enzymic action is obvious. It may also speed up the process of penetration and in certain cases serve as the chief, if not the sole, means of invasion, but it plays only a minor and nonessential part in the invasion of the tomato fruit cells by *Oospora lactis* and no doubt acts only in a secondary capacity in most cases. This is fortunate, for if the development of disease-resistant fruits depended upon thickness of walls, quality and resistance would often be diametrically opposed.

Since *Oospora* passes through the cellulose walls of two adjoining fruit cells without attaching itself to the wall or protoplasm and not infrequently makes an opening larger than the filament without causing a depression in the wall of the occupied cell, nor lateral cracks, or ruptures, in the wall of the invaded cell, its means of penetration can not be ascribed to pressure. The only other known means by which a fungus can make an opening of this description is by the use of an enzyme, such as cellulase, which has been isolated from fungi.

There are features in the penetration of cell walls by some fungi, especially species of the genus *Pythium*, which cast considerable doubt on the penetration of cell walls by pressure. In his study of *Pythium gracile*, Ward observed that an oospore which had germinated at some little distance from a cress seedling, produced as it grew several bends in its germ tube and passed around a small algal cell at right angles before reaching a cress cell. On coming into contact with this cress cell its "apex became closely pressed against the cuticle, apparently lifting the whole hypha slightly in the process," evidently a result of pressure, but thereafter made no further movement nor change in its position as it produced a small hole in the cuticle and cellulose wall, passed through, and enlarged to normal size within the cell. As this fungus filament consisted of a single cell, the pressure within it was equally distributed. If the pressure had been sufficient to penetrate the host cell wall, which was many times as thick as the fungus wall, it would have straightened the fungus filament. As a matter of fact, however, it did not straighten a single bend nor change its position in the least, although such filaments bend easily, even by the motion of delicate water currents. Moreover, if pressure had caused the penetration of the wall, the fungus would have made a hole as large as its filament instead of a small hole, for the pressure on every unit area of its wall surface was equal. A more probable cause of this type of penetration is that cellulase is formed solely at a single point on the tip of the filament.

In view of the foregoing evidence, it would seem that the pressure theory of cell wall penetration by fungi is not so well supported as the enzym theory.

#### DISTRIBUTION OF THE FUNGUS

As this physiological form of *Oospora lactis* has been isolated frequently from tomato fruits shipped from the Gulf States, it is probably common in at least several of the Southern States. It is difficult from the reports of the Bureau of Markets' inspectors to estimate how often it really occurs in shipped tomatoes, as they report all such rots as "soft rot." It was found to be common at the Government Experimental Farm, Arlington, Va., and in gardens in the vicinity of Washington, D. C., where no doubt it has carried on its parasitic activities for some time, but has been overlooked because of the similarity of the rots produced by it and *Bacillus carotovorus* and the not infrequent association of the two organisms in the same fruits.

#### POSSIBLE MEANS OF CONTROL

As this fungus infects tomato fruits quite readily between 9° and 32° C. and can infect them at temperatures ranging from 2° to 37.5°, the practicability of controlling it by means of low temperatures seems doubtful.

Some experiments were made to determine the effects of antiseptics on the control of this organism. The fungus was immersed for 30 minutes in an aqueous solution of the antiseptic and transferred to sterile carrot decoction, in which, if it were still viable, it grew readily. The results are summarized in Table III.

TABLE III.—Effect of antiseptics on the viability of *Oospora lactis parasitica*

Antiseptic.	Concentration of solution.		Subsequent growth in carrot decoction.
	Antiseptic.	Water.	
Chlorid of lime.....	I	40	None.
Potash alum.....	(a)	(a)	Considerable.
Potassium permanganate.....	I	400	None.
Copper sulphate.....	(a)	(a)	Good.
Mercuric chlorid.....	I	5, 000	None.
Formaldehyde, 37 per cent.....	I	300	Do.
Water.....			Abundant.

a Saturated.

Immersing the fungus for 30 minutes in an aqueous solution of chlorid of lime 1:40, potassium permanganate 1:400, formaldehyde (37 per cent) 1:300, or mercuric chlorid 1:5000, prevented its subsequent growth, but a similar treatment with a saturated solution of potash alum or copper sulphate was ineffective. The resistance of this fungus to copper sulphate—a fungicide of wide use—is surprising. Although the experiments with this treatment were repeated several times, only negative action was obtained.

Some experiments were also made on the use of the antiseptics as washes. Tomato fruits varying in maturity from green to ripe were lightly pricked in several places and submerged for 5 minutes in an aqueous suspension of *Oospora* spores, then drained, washed in an antiseptic solution for 30 minutes, and placed in moist chambers. From 20

to 40 pricked fruits, 8 unpricked fruits, and a number of controls equal to the number of treated fruits were used in each treatment. The results are summarized in Table IV.

TABLE IV.—Effect of washing green to ripe tomato fruits for 30 minutes in an antiseptic to control watery-rot

Fungicide.	Strength of solution; parts antiseptic to water.	Pricked fruit infected 6 days after treatment.	Unpricked fruit infected 12 days after treatment.
		Per cent.	Per cent.
Potash alum.....	1:50	31	25
Do.....	1:40	30	.....
Chlorid of lime.....	1:50	20	.....
Do.....	1:40	14	12½
Formaldehyde (37 per cent).....	1:300	30	0
Do.....	1:240	22	0
Control.....	.....	57	37½

The percentage of infected fruits is higher in the pricked than in the unpricked series. Moreover, it is higher than in shipped fruits. As shipped fruits usually become infected through the stem scar instead of through the epidermis, they are probably comparatively free from punctures and therefore compare more nearly with the unpricked series. Formaldehyde and chlorid of lime (calcium hypochlorite) caused considerable reduction in the percentage of infected fruits. It is quite likely that sodium hypochlorite, which is a cheap convenient solution, would give more effective results than chlorid of lime. As the latter is partly insoluble it should, if used on a commercial scale, be dissolved in a separate tank so the clear liquid can be drawn off for use, or dissolved in a washing tank having a removable perforated sheet a few inches above the bottom to prevent the solid particles from adhering to the fruits.

The lower effectiveness of these antiseptics in the presence of fruits as compared with their effect on free spores (Table III) is probably due to chemical reaction of the germicides with substances on the surface of the fruits, which reduces their strength.

The economical use of a fungicide for washing tomato fruits should not be measured by its effect on a single rot, for if it controls one, it will give at least partial control of several others that cause heavy loss during shipment. The selection of a solution for this purpose should therefore be based on its control of this whole group of rots as well as on its cheapness and ease of handling.

#### SUMMARY

(1) A new rot of tomato fruits, closely resembling the rot caused by *Bacillus carotovorus*, has been common since the spring of 1921 in tomatoes shipped from the Gulf States. It is also prevalent in the vicinity of Arlington, Va., and Washington, D. C.

(2) The rot is characterized by dark-colored water-soaked areas which start in the stem scar and spread slowly toward the blossom end of the fruit without the production of a pronounced odor.

(3) The causal organism is a physiological form of *Oospora lactis*, which, though incapable of penetrating the epidermis, usually enters the fruits through the stem scar, but may enter through any place not cov-

ered by the cuticle, such as wounds, cracks, and punctures. It shows a little preference for ripe fruits, but infects green fruits quite readily. It invades the cells, destroys their protoplasmic contents, and causes loss of cell coherence through a slow dissolution of the middle lamellae of the cell walls.

(4) The minimum temperature for the germination of its spores, growth of its mycelium, and its infection of tomato fruits is approximately  $2^{\circ}\text{C}$ ., the optimum  $30^{\circ}$ , and the maximum between  $37.5^{\circ}$  and  $38.5^{\circ}$ .

Immersing the fungus spores (cells) in an aqueous solution of an anti-septic for 30 minutes and transferring them to sterile carrot decoction had the following effects on their viability: Chlorid of lime 1:40, potassium permanganate 1:400, formaldehyde (37 per cent) 1:300, and mercuric chlorid 1:5,000, no growth; potash alum or copper sulphate in saturated solution, considerable growth. The percentage of tomato fruits infected after inoculation with this fungus was considerably reduced by washing them for 30 minutes in an aqueous solution of chlorid of lime 1:40 or formaldehyde (37 per cent) 1:240. It would seem from these results that an effective wash could be developed for the control of a large number of these rots during shipment.

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# PLATE 1

Sections of a 2-day-old spot of a green tomato fruit affected by watery-rot (*Oospora lactis parasitica*).

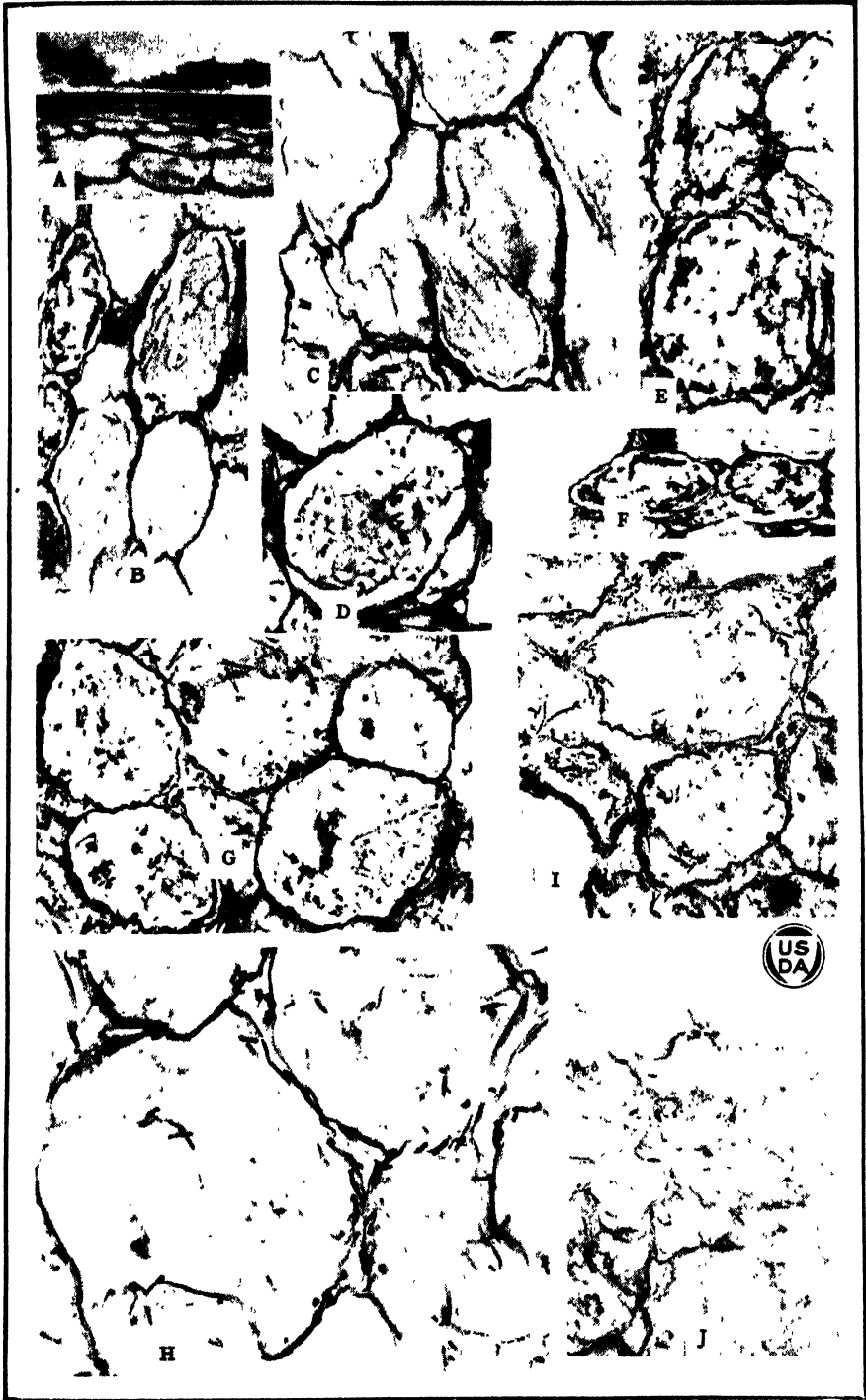
A.—Masses of the fungus lying on the surface unable to penetrate the cuticle.

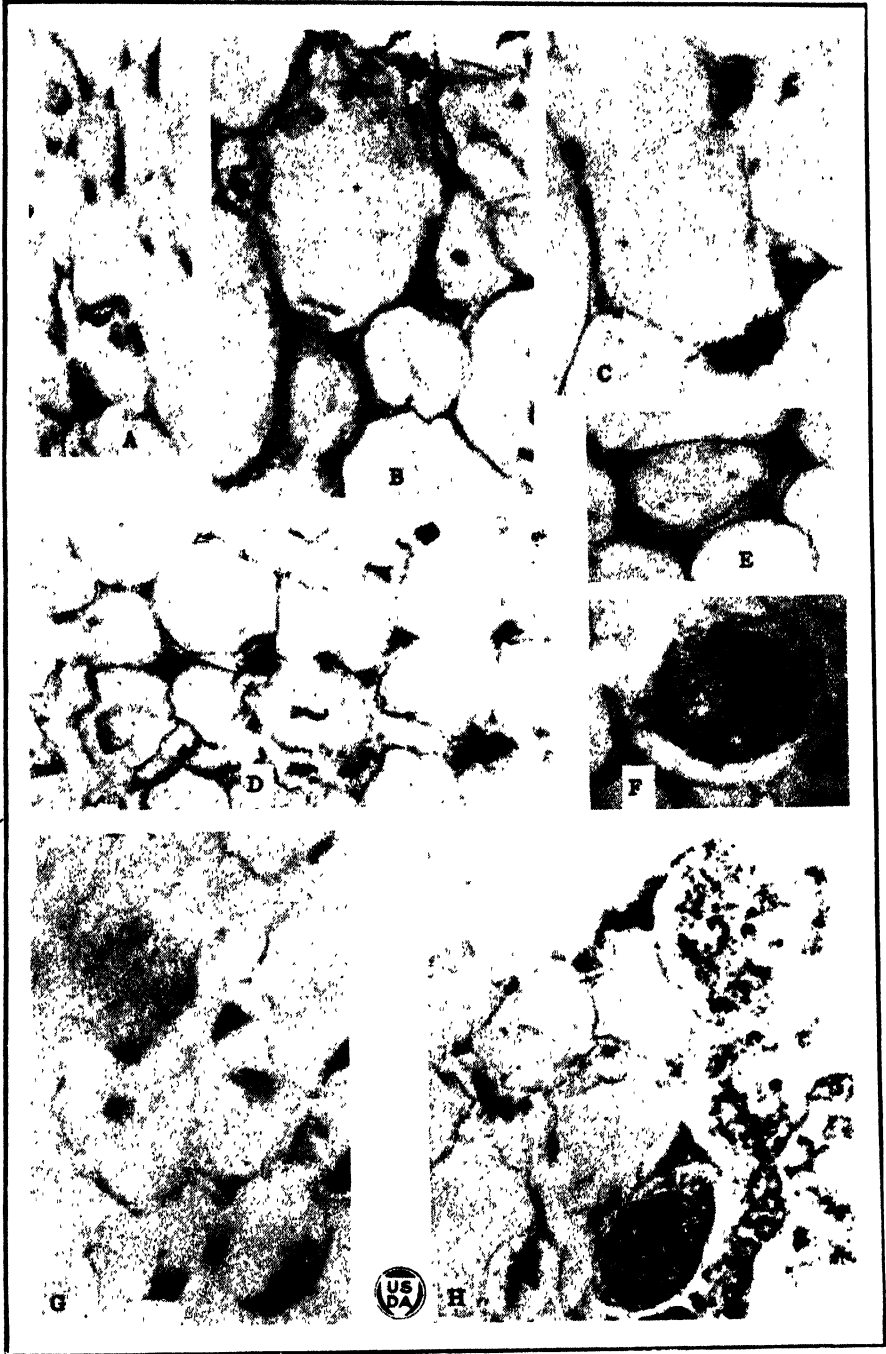
B-G.—Consumption of the protoplasm by the fungus; B-C, early stages; D, medium early stage; E-G, late stages.

H.—Cells beginning to lose their coherence after destruction of their protoplasm.

I.—A more complete stage of cell separation than shown in H.

J.—Very late stage. Protoplasmic contents completely destroyed; cells free; walls thin, irregular, and inconspicuous.





## PLATE 2

Sections of a tomato fruit infected by *Bacillus carotovorus*.

A.—Early stage of invasion; bacteria confined to the intercellular spaces.

B-D.—Medium early stages. B-C.—Intercellular spaces enlarging through dissolution of the middle lamellae of the adjoining cell walls; bacteria beginning to invade the cells.

D.—Cells losing their turgidity and coherence; middle lamellae dissolved.

E.—Bacterial invasion of a firmly attached cell.

F-H.—Late stages. Cells occupied by the bacteria.



### PLATE 3

#### *Oospora lactis parasitica*

A-D.—Types of branches. A.—Short branches which arise near the septa at an angle of about  $45^{\circ}$  with the filament and break up into more or less rounded, irregularly shaped cells which function as spores. B-D.—Long branches which bear lateral branches of type A, but also break up into numerous cells capable of immediate germination.

E-F.—Segmented branches of type A; also early stages in the segmentation of the central filament.

G.—Order of segmentation in branch of type A.

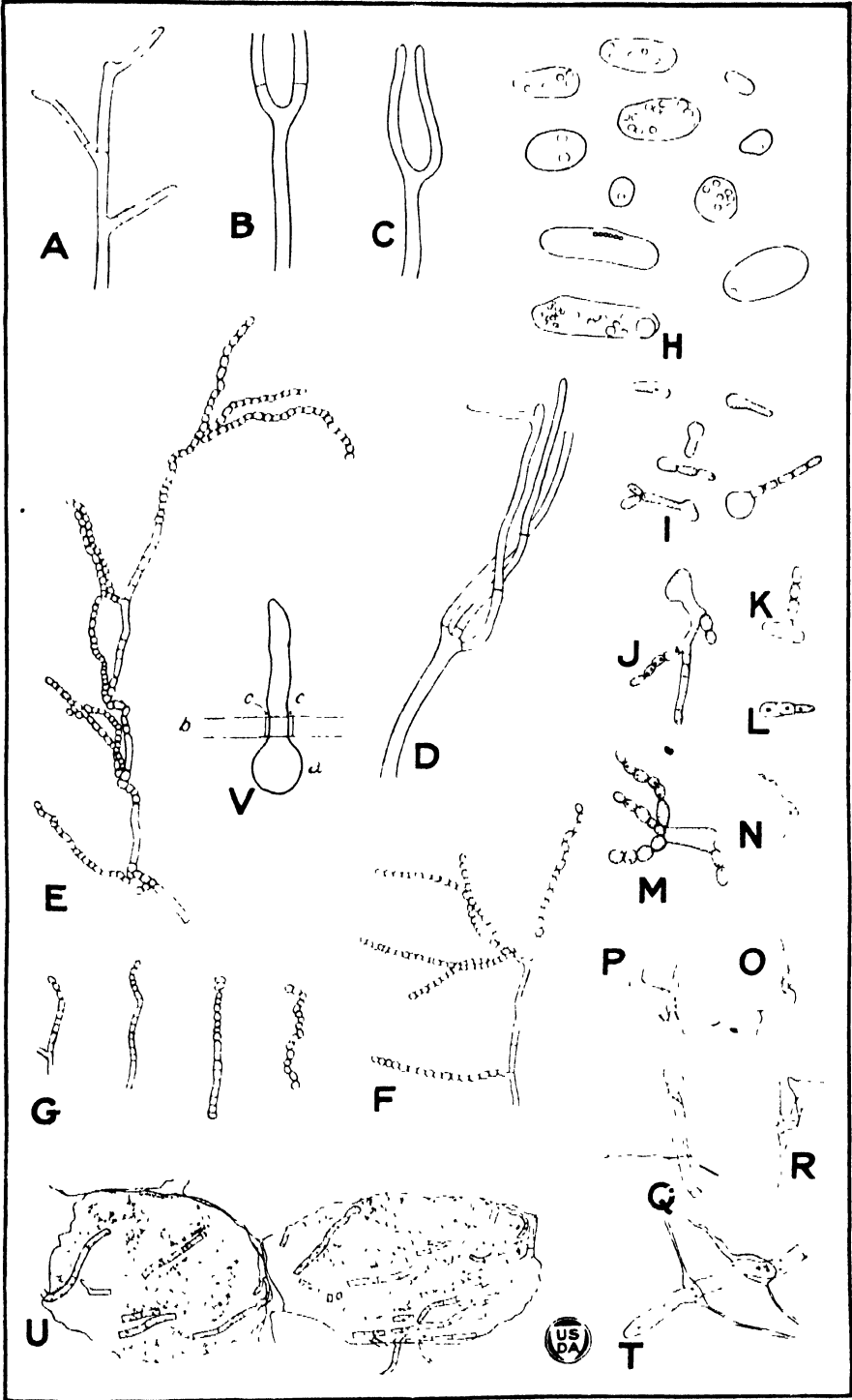
H.—Highly magnified detached cells of lateral branches and central filament.

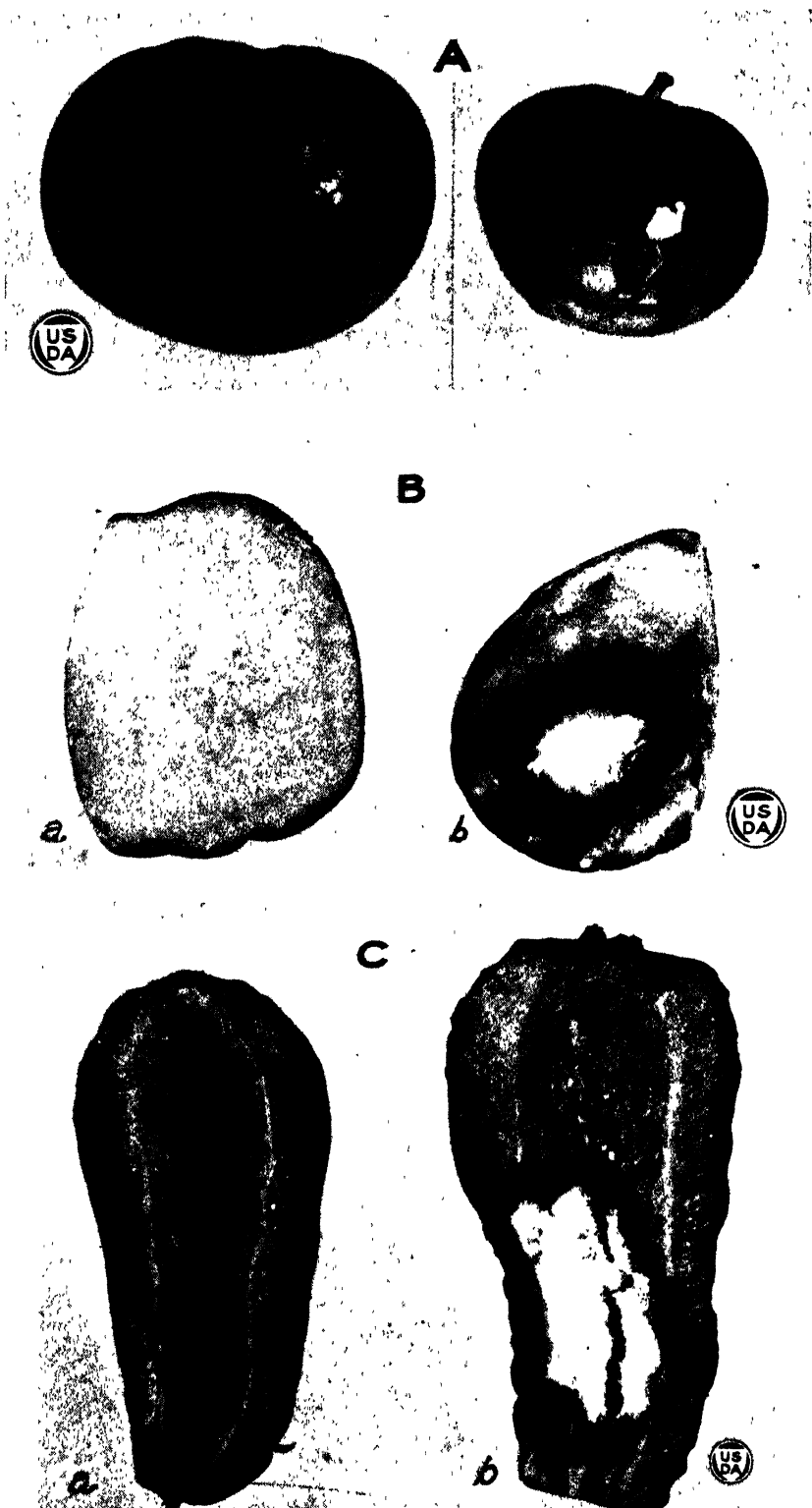
I-M.—Germinating detached cells.

N-T.—Penetration of irregular, wavy, somewhat separated walls of partly destroyed cells by tips of growing hyphae.

U.—Cells of tomato fruit tissue containing hyphae surrounded by digestion vacuoles.

V.—Penetration of the cell wall of a tomato fruit cell by a germinating spore. (a) Germinating spore. (b) Cell wall. (c) Hole made in the wall by the germ tube. Somewhat diagrammatic.





#### PLATE 4

A.—Watery-rot of tomato fruits produced by *Oospora lactis parasitica*. The fruits were inoculated in needle punctures below the stem scar and kept in a moist atmosphere.

B.—Halved potato tubers. (a) Inoculated with *Oospora lactis parasitica*; (b) inoculated with *Bacillus carotovorus*.

C.—Halved carrot roots. (a) Pricked with a sterile needle; (b) inoculated with *Oospora lactis parasitica* isolated from watery-rot lesions of tomato fruit.



# INFLUENCE OF THE ABSOLUTE REACTION OF A SOIL UPON ITS AZOTOBACTER FLORA AND NITROGEN FIXING ABILITY<sup>1</sup>

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## INTRODUCTION

In a preliminary report the writer (2)<sup>2</sup> called attention to the apparent close correlation existing between the absolute reaction of a soil extract and the presence of *Azotobacter* in the soil. The data presented in this report showed that when the hydrogen-ion concentration of the soil extract exceeded  $1 \times 10^{-6}$  the soil, with very few exceptions, failed to initiate the growth of *Azotobacter* when introduced into a suitable mannite culture solution. On the other hand, when the hydrogen-ion concentration of the soil extract was less than  $1 \times 10^{-6}$  similar cultures almost always developed typical *Azotobacter* films. The data, though meager, indicated that the maximum hydrogen-ion concentration endured by *Azotobacter* in soils was near that represented by a  $P_H$  of 6.

In the preliminary report only 90 soils were examined and the hydrogen-ion concentration of the soil extract was determined by the Clark and Lubs colorimetric method as modified for soils by Gillespie (7). Some investigators place little credence in the colorimetric method for determining hydrogen-ion concentrations, particularly in a medium as complex as a soil extract. Gillespie, however, found only slight differences in the hydrogen-ion concentration of soil extracts determined by this method and in suspensions of the same soils determined electrometrically. Since the publication of the preliminary report 418 soils from widely varying localities and conditions have been subjected to similar examinations, the resulting data being the basis of this paper. The hydrogen-ion concentration of these soils has been determined colorimetrically upon an extract and electrometrically upon a suspension of the soil.

The soils of Series I were collected within a few miles of the station either by the writer or one of his assistants. These soils represent practically all types and conditions of soil found in this immediate vicinity. Those of Series II were also collected near the station and in many instances at or near the point where soils of Series I were taken. These collections were made 2 years later to serve as a control on the first examinations. Where soils are duplicates of former samples, it has been indicated in Table VII by recording the former numbers. The soils of Series III were collected, as indicated in Table IX, from different counties in the State of Kansas by the county agents. Sterile containers were sent out and requests were made to collect soils representative of conditions in the area covered. The directions called for removing the inch or two of surface soil and collecting from at least four points within a short distance of each other in order to obtain as far as possible a representative sample of the immediate area. In Series IV the soils

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 937-938.

were obtained from a number of different States. These were secured, in most instances, through the various experiment stations. The collector was requested to send samples, where possible, from alkaline or limed, and acid or unlimed, adjacent soils.

#### METHODS

Soon after the soils reached the laboratory they were well mixed and four 300 cc. Erlenmeyer flasks containing 50 cc. of mannite cultural solution were inoculated from each soil. Ten cubic centimeters of a suspension prepared by shaking 1 part of soil with 2 parts of sterile water was used as an inoculum. The suspension was allowed to stand a few minutes to let the heavier soil particles settle out. Two of the cultures were immediately sterilized in the autoclave to act as controls on total nitrogen determinations.

The culture medium employed had the following composition:

Magnesium sulphate.....	0.2 gm.
Di-basic potassium phosphate.....	0.2 gm.
Sodium chlorid.....	0.5 gm.
Ferric chlorid.....	Trace.
Calcium chlorid.....	Trace.
Mannite.....	20.0 gm.
Distilled water.....	1,000.0 cc.

This medium was rendered slightly alkaline to phenolphthalein with sodium hydroxid. In all experiments, except those reported in Table V, a small quantity of sterile calcium carbonate was added to each culture flask before inoculating. In all cases the cultures were incubated at room temperature for 3 weeks, after which total nitrogen determinations were made according to the modification of the Kjeldahl method suggested by Latshaw (10). The quantities of nitrogen reported represent the average of duplicate cultures after deducting the average of duplicate controls.

During the incubation period frequent examinations were made both macroscopically and microscopically, to ascertain the character of the growth. When "no film" is reported, no growth resembling *Azotobacter* took place during the first two weeks of incubation. After approximately 2 weeks of incubation a heavy fungus growth usually appeared, especially where no *Azotobacter* growth, or a nontypical *Azotobacter* film developed. After the development of a fungus film the growth became so complex that it was difficult to detect *Azotobacter* either macroscopically or microscopically. Such was the appearance of those cultures in which *Azotobacter* is reported as questionable. It is believed that the results would have been more consistent and striking if incubation had been reduced to 2 weeks. This would have avoided, to a large extent, the complications arising from the growth of fungi.

The microscopic examinations were made by placing on a slide a loop of that part of the surface growth which appeared most characteristic of *Azotobacter*, covering with cover glass, and examining with the 1/6 objective. If typical *Azotobacter* were present in appreciable numbers, the picture was so striking as to be almost unmistakable. If *Azotobacter* are not present in a soil in sufficient numbers and vigor to develop a visible film or to produce sufficient growth to be observed microscopically by the methods employed, it is questionable whether they are of any significance in the nitrogen economy of a soil.

In this examination of soils for *Azotobacter* it will be noted that three methods of detecting their presence were employed—the formation of a film, the microscopic examination, and the quantity of nitrogen fixed. Under the heading "*Azotobacter*" in Tables V, VII, IX, and XI a + (plus) sign has been placed where, in the opinion of the writer, the major evidence indicated the existence of a vigorous *Azotobacter* flora in the original sample of soil and a - (minus) sign where the evidence did not indicate the presence of a vigorous *Azotobacter* flora.

The colorimetric hydrogen-ion determinations were made by the Clark and Lubs method as modified for soils by Gillespie. The soils were ground to pass a 40-mesh sieve and a weighed quantity mixed with five times its weight of water, shaken well and centrifugalized until the supernatant liquid was practically clear. The water used in the preparation of the soil extract and suspension was freshly distilled from a mixture of sulphuric acid and potassium dichromate into a flask containing barium hydroxid. From this it was distilled into a third empty flask and again distilled. The  $P_H$  of water thus obtained was from 5.7 to 6.0 and was affected by the minutest trace of acid or alkali. All glassware coming in contact with the soil extract was washed in this water. Buffer solutions were prepared according to Clark and Lubs (1) and were checked, and adjusted if necessary, at frequent intervals on a Leeds and Northrup type K potentiometer.

Electrometric hydrogen-ion concentrations, or differences in potential between the soil solution and the hydrogen electrode, were made by using a Leeds and Northrup type K potentiometer in connection with saturated KCl—calomel and hydrogen electrodes similar to the one described by Hildebrand (9). The ratio of soil to water used was the same as employed in colorimetric determinations; i. e., 1 to 5. Six hydrogen electrodes were connected by switches to the potentiometer so that six samples could be run at the same time. Hydrogen was bubbled through the cells continuously at a rather rapid rate, the cells being constantly shaken. A maximum difference in potential was usually recorded in 10 to 30 minutes, after which the difference decreased very slowly. The length of time required to reach the maximum reading apparently depended, other things being equal, upon the rate of flow of hydrogen. The influence of the rate of flow of hydrogen upon the length of time necessary to obtain maximum difference of potential is illustrated in the data presented in Tables I and II. Neutral or alkaline soils usually required a longer time to reach the maximum difference in potential, and the agreement between duplicates was not, as a rule, as close as it was with acid soils.

TABLE I.—Time required for electrodes to record maximum difference in potential; hydrogen passed over electrodes slowly (readings recorded as millivolts)

Soil No.	Time (in minutes).													
	15	20	30	35	40	45	50	55	60	70	80	90	95	100
376.....	613	.....	625	.....	.....	.....	655	.....	673	683	687	690	.....	<sup>a</sup> 693
376.....	618	.....	654	.....	.....	.....	678	.....	685	690	694	695	.....	<sup>a</sup> 696
380.....	503	.....	515	.....	.....	554	563	565	<sup>a</sup> 566	.....	.....	.....	.....	.....
380.....	517	.....	540	.....	.....	563	<sup>a</sup> 566	566	566	.....	.....	.....	.....	.....
381.....	.....	520	.....	523	.....	<sup>a</sup> 524	.....	524	.....	.....	.....	.....	.....	.....
381.....	.....	500	.....	524	.....	<sup>a</sup> 526	.....	526	.....	.....	.....	.....	.....	.....
410.....	.....	633	.....	.....	642	.....	.....	670	.....	695	.....	.....	<sup>a</sup> 703	703
410.....	.....	654	.....	.....	691	.....	.....	697	.....	<sup>a</sup> 702	.....	.....	702	700

<sup>a</sup> Maximum reading.



TABLE II.—Time required for electrodes to record maximum difference in potential; hydrogen passed over electrodes rapidly (readings recorded as millivolts)

Soil No.	Time (in minutes).						
	5	10	15	20	25	30	35
74.....	678	688	<sup>a</sup> 691	690	690	.....	.....
74.....	668	681	680	<sup>a</sup> 682	682	.....	.....
75.....	674	683	<sup>a</sup> 688	688	688	.....	.....
75.....	670	685	688	690	<sup>a</sup> 692	.....	.....
76.....	583	<sup>a</sup> 587	586	585	584	.....	.....
76.....	590	<sup>a</sup> 592	591	590	588	.....	.....
24.....	648	.....	<sup>a</sup> 675	.....	674	.....	.....
24.....	630	.....	<sup>a</sup> 667	.....	664	.....	.....
26.....	662	.....	<sup>a</sup> 670	.....	666	.....	.....
26.....	665	.....	<sup>a</sup> 670	.....	670	.....	.....
28.....	<sup>a</sup> 690	.....	690	.....	686	.....	.....
28.....	682	.....	680	.....	<sup>a</sup> 688	.....	.....
209.....	<sup>a</sup> 576	574	573	.....	.....	.....	.....
209.....	535	570	<sup>a</sup> 577	.....	.....	.....	.....
210.....	540	<sup>a</sup> 546	546	.....	.....	.....	.....
210.....	536	546	<sup>a</sup> 550	.....	.....	.....	.....
191.....	.....	.....	<sup>a</sup> 562	.....	562	.....	560
191.....	.....	.....	564	.....	<sup>a</sup> 566	.....	564
192.....	.....	.....	<sup>a</sup> 562	.....	560	.....	558
192.....	.....	.....	560	.....	<sup>a</sup> 564	.....	564
265.....	.....	.....	<sup>a</sup> 515	.....	515	514	.....
265.....	.....	.....	<sup>a</sup> 515	.....	515	514	.....
166.....	575	.....	.....	<sup>a</sup> 588	.....	586	.....
166.....	565	.....	.....	<sup>a</sup> 586	.....	584	.....
38.....	556	.....	.....	<sup>a</sup> 563	.....	562	562
38.....	525	.....	.....	557	.....	<sup>a</sup> 561	560
8.....	548	.....	.....	582	.....	<sup>a</sup> 585	585
8.....	548	.....	.....	577	.....	584	<sup>a</sup> 585

<sup>a</sup> Maximum reading.

The platinum electrodes used were coated with platinum black and tested on a standard acetate solution before using. Several determinations could usually be run with one coating of platinum black. Duplicate samples of soil were always run and as a rule the results agreed within 10 millivolts. If the disagreement were much greater than this, the sample was again run. Sharp and Hoagland (13) state that "Duplicate determinations on soil suspension usually agreed within 0.01 to 0.02 volt." Plummer (11) says "Duplicate readings on the same sample of soil could easily be read to 0.02 volt," while "It was almost impossible to get such closely agreeing results as 0.02 volt with different samples of the same soil." The data presented in Tables I, II, III, and IV illustrate the millivolt readings of duplicate samples run on different electrodes. In Table IV are shown the millivolt readings of samples of the same soil run on different dates and also the slight effect upon the reading of varying the ratio of soil to water.

TABLE III.—Effect on electrode readings of varying the method of saturating the electrode and suspension with hydrogen

Soil No.	Hydrogen run over electrode continuously.					
	Electrode number—					
	1	2	3	4	5	Average.
401.....	615	608	595	601	619	608
362.....	557	550	552	553	554	553
378.....	542	544	541	541	538	541
377.....	545	542	549	541	551	546

Soil No.	1,000 cc. hydrogen run over electrode.					
	Electrode number—					
	1	2	3	4	5	Average.
401.....	<sup>a</sup> 597	<sup>a</sup> 611	<sup>a</sup> 611	<sup>a</sup> 615	611	609
362.....	550	550	550	550	.....	550
378.....	543	542	530	540	538	539
377.....	550	<sup>a</sup> 530	<sup>a</sup> 537	<sup>a</sup> 538	554	542

Soil No.	1,000 cc. hydrogen run over electrode, then run continuously.					
	Electrode number—					
	1	2	3	4	5	Average
401.....	629	620	622	624	614	622
362.....	553	553	557	555	.....	554
378.....	543	543	533	543	530	538
377.....	555	554	550	552	552	553

<sup>a</sup> These samples apparently did not have sufficient hydrogen passed through to saturate the electrode and suspension, as is evidenced by the increased reading when further passage of hydrogen took place.

TABLE IV.—Difference in potential of same soil determined on different dates, effect of varying ratio of soil to water, and variations in difference in potential as determined with five different electrodes

Soil No.	Ratio of soil to water	Electrode number—					
		1	2	3	4	5	Average
390 <sup>a</sup> .....	1 to 5.....	534	544	541	539	532	537
390 <sup>b</sup> .....	1 to 5.....	538	540	539	532	536	537
369.....	1 to 5.....	678	679	677	678	676	678
395.....	1 to 5.....	530	528	528	528	529	529
395.....	1 to 10.....	536	533	536	536	539	536
387.....	1 to 5.....	493	494	493	493	493	493
387.....	1 to 10.....	495	494	494	495	494	494
384.....	1 to 5.....	650	653	650	652	652	651
397.....	1 to 5.....	563	566	567	566	566	566

<sup>a</sup> April 5.

<sup>b</sup> May 31.

In converting volt readings into  $P_H$  use has been made of the tables prepared by Schmidt and Hoagland (12) adding 91 millivolts to the readings to convert them into N/10 KCl-calomel electrode readings. Some investigators regard the difference in potential between saturated and N/10 KCl-calomel electrodes to be of a value other than 91 millivolts. To convert the  $P_H$  values here recorded into those of any other difference in potential between saturated and N/10 KCl-calomel electrodes, it is only necessary to add or subtract, as the case may be, 0.017 from the figure here recorded for each millivolt above or below 91. All determinations were made at room temperature, and Schmidt and Hoagland temperature correction factors were used to convert room temperature readings into 25° C. readings.

Hydrogen was purchased in cylinders and washed through a saturated solution of mercuric chlorid, alkaline potassium permanganate solution, alkaline pyrogalllic acid solution, and distilled water before entering the hydrogen electrode cell. The connection between the calomel and hydrogen electrodes was made through a glass stopcock. The end of this immersed in the soil suspension was drawn out to a capillary opening and the cock was kept closed during the determination. Between successive determinations, however, the connection was refilled with fresh saturated potassium chlorid.

## RESULTS

Data relative to the type of growth and the quantity of nitrogen fixed in culture media inoculated from soils, together with the absolute reaction of the soil extract determined colorimetrically and the soil suspension determined electrometrically of 418 soils, are recorded in Tables V, VII, IX, and XI. In Tables VI, VIII, X, and XII some of the data have been rearranged to show the possible association, or correlation, existing between the hydrogen-ion concentration of the soil solution and the presence or absence of *Azotobacter* in the soils. In these various tables under the heading "*Azotobacter*" a + (plus) sign has been recorded to indicate the presence of *Azotobacter* and a - (minus) sign to indicate the absence of *Azotobacter* in the soils. These data are not absolute. Sometimes it was very difficult to differentiate between the presence or absence of *Azotobacter*. There are a few instances where an *Azotobacter* film developed in only one of duplicate samples, or where either the film or *Azotobacter* cells were questionable, accompanied by poor fixation of nitrogen, that *Azotobacter* have been recorded as absent. On the other hand, there are a few instances where similar conditions were accompanied by good fixation of nitrogen that have been recorded as containing *Azotobacter*. An effort has been made to weigh the evidence in questionable cases as carefully as possible and record *Azotobacter* as present if it were believed that the data indicated the presence of an active *Azotobacter* flora, and as absent if the available data did not indicate the presence of such a flora.

It should be borne in mind that the chance for contamination of *Azotobacter* free soils with *Azotobacter* was not entirely eliminated. Especially was this true in Series III and IV, for nearly all of the soils in these two series were collected by men inexperienced in bacteriological technic and were shipped long distances. Sometimes one or more of the individual containers were broken open upon arrival at the laboratory.

Even in the laboratory the possibility of contamination could not be entirely eliminated. What influence such contamination may have had upon the development of *Azotobacter* from soils that normally contained no *Azotobacter* is not known.

Again, it has been shown (3) that *Azotobacter* can exist for varying lengths of time in soils that will not support them indefinitely. Under natural conditions, if *Azotobacter* exists in the proximity of soils not containing them, almost constant inoculation due to wind, rain, animals, cultivation, etc., is inevitable. The length of time that such introduced organisms may remain in a viable condition apparently depends upon the intensity of the unfavorable influences. It is, therefore, highly probable that *Azotobacter* may frequently be isolated from soils in which they will not function or even exist for a very long period.

On the other hand, little is known as to how many *Azotobacter* are necessary to initiate the development of a visible film in laboratory culture media. Unpublished data indicate that appreciable numbers are essential to the development of a characteristic film. Also, nothing is known as to how rapidly they may disappear from a sample of soil removed from its natural environment. It is possible, therefore, that *Azotobacter* may be present in relatively large numbers in a soil and escape observation by the methods employed in these investigations.

In comparing the reaction with the presence or absence of *Azotobacter*, and in obtaining a mathematical expression for this association or correlation (the association coefficient), the soils have been divided into two groups; those with a  $P_H$  of 6.0 or above and those more acid than  $P_H$  6.0. This division point has been chosen more or less arbitrarily, though the data here presented indicate that it is not far from absolute. The association coefficient was obtained by the use of Yule's (14) association coefficient formula, as mentioned elsewhere (5). Association coefficients have been calculated both from the data secured by the colorimetric and the electrometric  $P_H$  determination. In analyzing the data presented here it will perhaps be best to examine those from each series of soils separately.

#### SERIES I

The data secured from an examination of the soils of Series I are presented in Table V, rearranged in part in Table VI, and summarized below. These soils together with those of Series II are all local soils and were, perhaps, collected and handled with more care than those of Series III and IV. Samples 1 to 19 were lost through error before the electrometric hydrogen-ion determinations were run. However, in the following summary the electrometric  $P_H$  of samples No. 1, 4, 5, 14, and 15 have been regarded as above  $P_H$  6.0 and all other soils below No. 20 as more acid than  $P_H$  6.0. These 19 soils could not be considered in obtaining the numerical average of colorimetric  $P_H$ . Likewise samples 85-90, for which no quantitative nitrogen determinations were made, were omitted from the calculations for average nitrogen fixed.

TABLE V.—Type of growth, nitrogen fixed, and reaction of soils of Series I

Soil No.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P <sub>H</sub> colorimetric.	P <sub>H</sub> electrometric.
			Mgm.			
1.....	Typical Azotobacter...	Typical Azotobacter...	10.3	+	6.9	.....
2.....	None .....	No Azotobacter .....	2.0	—	5.4	.....
3.....	do. ....	do. ....	3.0	—	5.6	.....
4.....	Typical Azotobacter...	Typical Azotobacter...	6.8	+	6.9	.....
5.....	do. ....	do. ....	5.4	+	7.1	.....
6.....	None .....	No Azotobacter .....	4.4	—	5.7	.....
7.....	do. ....	do. ....	4.4	—	5.7	.....
8.....	do. ....	do. ....	4.6	—	5.6	.....
9.....	do. ....	do. ....	4.7	—	5.5	.....
10.....	do. ....	do. ....	—0.6	—	5.6	.....
11.....	do. ....	do. ....	1.7	—	5.8	.....
12.....	do. ....	do. ....	4.5	—	5.9	.....
13.....	do. ....	do. ....	3.2	—	5.6	.....
14.....	Typical Azotobacter...	Typical Azotobacter...	8.5	+	7.4	.....
15.....	do. ....	do. ....	10.3	+	7.4	.....
16.....	None .....	No Azotobacter .....	4.0	—	5.6	.....
17.....	do. ....	do. ....	3.0	—	5.5	.....
18.....	do. ....	do. ....	3.7	—	5.6	.....
19.....	do. ....	do. ....	4.5	—	5.7	.....
20.....	do. ....	do. ....	4.7	—	5.4	5.10
21.....	do. ....	do. ....	4.7	—	5.6	5.05
22.....	do. ....	do. ....	4.7	—	5.6	5.27
23.....	do. ....	do. ....	4.7	—	5.6	5.10
24.....	do. ....	do. ....	4.2	—	5.6	4.90
25.....	Typical Azotobacter...	Typical Azotobacter...	6.4	+	7.0	6.45
26.....	do. ....	do. ....	9.9	+	6.6	6.13
27.....	do. ....	do. ....	7.5	+	6.1	5.88
28.....	do. ....	do. ....	9.5	+	6.2	5.86
29.....	do. ....	do. ....	8.8	+	7.6	7.27
30.....	None .....	No Azotobacter .....	4.1	—	5.6	5.39
31.....	Typical Azotobacter...	Typical Azotobacter...	10.6	+	7.5	7.71
32.....	None .....	No Azotobacter .....	5.6	—	5.9	5.85
33.....	do. ....	do. ....	4.0	—	6.7	6.56
34.....	do. ....	do. ....	4.5	—	6.8	6.74
35.....	Typical Azotobacter...	Typical Azotobacter...	6.8	+	7.6	7.62
36.....	do. ....	do. ....	9.2	+	6.0	6.12
37.....	None .....	No Azotobacter .....	5.4	—	6.2	6.07
38.....	Nontypical <sup>a</sup> .....	Azotobacter present <sup>a</sup> .....	3.9	—	5.6	5.59
39.....	do. ....	do. ....	8.6	+	6.1	6.05
40.....	do. ....	do. ....	8.1	+	7.0	6.72
41.....	do. ....	Typical Azotobacter...	7.6	+	6.0	5.98
42.....	do. ....	do. ....	8.3	+	7.4	7.05
43.....	Typical Azotobacter...	do. ....	9.6	+	7.7	8.75
44.....	Nontypical .....	do. ....	9.9	+	7.5	7.71
45.....	Typical Azotobacter...	do. ....	10.1	+	7.4	7.45
46.....	None .....	No Azotobacter .....	1.0	—	5.9	6.03
47.....	Typical Azotobacter...	Typical Azotobacter...	10.0	+	7.4	7.52
48.....	Nontypical .....	do. ....	6.6	+	6.4	6.52
49.....	None .....	No Azotobacter .....	2.7	—	5.5	5.03
50.....	do. ....	do. ....	4.0	—	5.8	5.32
51.....	do. ....	do. ....	3.2	—	5.3	5.41
52.....	Nontypical .....	Azotobacter present .....	5.5	+	7.3	7.27
53.....	Typical Azotobacter...	Typical Azotobacter...	9.0	+	7.7	7.79
54.....	Nontypical .....	do. ....	7.4	+	6.0	6.18
55.....	Typical Azotobacter...	do. ....	8.7	+	7.5	7.50
56.....	Nontypical .....	do. ....	7.2	+	7.4	7.18
57.....	Typical Azotobacter...	do. ....	7.6	+	7.5	7.10

<sup>a</sup> P<sub>H</sub> not determined electrometrically.

TABLE V.—Type of growth, nitrogen fixed, and reaction of soils of Series I—Continued

Soil No.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P <sub>N</sub> colorimetric.	P <sub>N</sub> electrometric.
			Mgm.			
58....	None.....	No Azotobacter.....	4.0	—	5.5	5.46
59....	Typical Azotobacter...	Typical Azotobacter....	10.6	+	7.4	7.49
60....	None.....	No Azotobacter.....	4.6	—	5.8	5.73
61....	do.....	do.....	3.7	—	5.5	5.51
62....	Typical Azotobacter...	Typical Azotobacter....	7.3	+	7.5	7.55
63....	do.....	do.....	11.0	+	7.4	7.43
64....	do.....	do.....	8.3	+	6.1	6.35
65....	None.....	No Azotobacter.....	5.2	—	5.7	5.94
66....	do.....	do.....	3.8	—	5.7	5.81
67....	do.....	do.....	4.1	—	5.5	5.32
68....	Nontypical.....	Typical Azotobacter....	6.9	+	6.1	6.05
69....	None.....	No Azotobacter.....	4.1	—	5.6	5.19
70....	Typical Azotobacter...	Typical Azotobacter....	8.7	+	6.8	6.93
71....	Nontypical.....	do.....	6.9	+	5.6	5.61
72....	do.....	do.....	6.8	+	7.0	6.72
73....	None.....	No Azotobacter.....	4.5	—	5.9	5.88
74....	Nontypical <sup>a</sup> .....	Typical Azotobacter <sup>a</sup> ...	8.1	+	7.4	7.25
75....	Typical Azotobacter...	do.....	6.1	+	7.5	7.30
76....	Nontypical <sup>a</sup> .....	do <sup>a</sup> .....	3.4	—	5.5	5.63
77....	None.....	No Azotobacter.....	4.2	—	5.6	5.34
78....	Nontypical.....	Typical Azotobacter....	5.5	+	7.7	7.54
79....	Typical Azotobacter...	do.....	7.6	+	7.7	8.48
80....	Nontypical.....	Azotobacter present....	9.2	+	7.6	7.84
81....	Typical Azotobacter...	Typical Azotobacter....	9.6	+	7.4	7.77
82....	do.....	do.....	8.0	+	7.7	7.60
83....	do.....	do.....	9.9	+	7.6	7.72
84....	do.....	do.....	9.5	+	7.5	7.47
85....	do.....	do.....	.....	+	7.5	7.62
86....	do.....	do.....	.....	+	7.5	7.60
87....	do.....	do.....	.....	+	7.8	7.59
88....	do.....	do.....	.....	+	6.9	6.88
89....	do.....	do.....	.....	+	7.5	7.76
90....	do.....	do.....	.....	+	7.3	7.06

<sup>a</sup> P<sub>N</sub> not determined electrometrically.

TABLE VI.—Correlation between reaction and presence of *Azotobacter* in soils of Series I

P <sub>H</sub> determined electrometrically.						P <sub>H</sub> determined colorimetrically.					
Soil No.	P <sub>H</sub> .	Azoto-bacter.	Soil No.	P <sub>H</sub> .	Azoto-bacter.	Soil No.	P <sub>H</sub> .	Azoto-bacter.	Soil No.	P <sub>H</sub> .	Azoto-bacter.
43.....	8.75	+	46...	6.03	—	87...	7.8	+	37...	6.2	—
79.....	8.48	+	41...	5.98	+	43...	7.7	+	27...	6.1	+
80.....	7.84	+	65...	5.94	—	53...	7.7	+	39...	6.1	+
53.....	7.79	+	27...	5.88	+	78...	7.7	+	64...	6.1	+
81.....	7.77	+	73...	5.88	—	79...	7.7	+	68...	6.1	+
89.....	7.76	+	28...	5.86	+	82...	7.7	+	36...	6.0	+
83.....	7.72	+	32...	5.85	—	29...	7.6	+	41...	6.0	+
31.....	7.71	+	66...	5.81	—	35...	7.6	+	54...	6.0	+
44.....	7.71	+	60...	5.73	—	80...	7.6	+	12 <sup>a</sup> ...	5.9	—
35.....	7.62	+	76...	5.63	—	83...	7.6	+	32...	5.9	—
85.....	7.62	+	71...	5.61	+	31...	7.5	+	46...	5.9	—
86.....	7.60	+	38...	5.59	—	44...	7.5	+	73...	5.9	—
82.....	7.60	+	61...	5.51	—	55...	7.5	+	11 <sup>a</sup> ...	5.8	—
87.....	7.59	+	58...	5.46	—	57...	7.5	+	50...	5.8	—
62.....	7.55	+	51...	5.41	—	62...	7.5	+	60...	5.8	—
78.....	7.54	+	30...	5.39	—	75...	7.5	+	6 <sup>a</sup> ...	5.7	—
47.....	7.52	+	77...	5.34	—	84...	7.5	+	7 <sup>a</sup> ...	5.7	—
55.....	7.50	+	50...	5.32	—	85...	7.5	+	19 <sup>a</sup> ...	5.7	—
59.....	7.49	+	67...	5.32	—	86...	7.5	+	65...	5.7	—
84.....	7.47	+	22...	5.27	—	89...	7.5	+	66...	5.7	—
45.....	7.45	+	69...	5.19	—	14 <sup>a</sup> ...	7.4	+	3 <sup>a</sup> ...	5.6	—
63.....	7.43	+	20...	5.10	—	15 <sup>a</sup> ...	7.4	+	8 <sup>a</sup> ...	5.6	—
57.....	7.30	+	23...	5.10	—	42...	7.4	+	10 <sup>a</sup> ...	5.6	—
75.....	7.30	+	21...	5.05	—	45...	7.4	+	13 <sup>a</sup> ...	5.6	—
29.....	7.27	+	49...	5.03	—	47...	7.4	+	16 <sup>a</sup> ...	5.6	—
52.....	7.27	+	24...	4.90	—	56...	7.4	+	18 <sup>a</sup> ...	5.6	—
74.....	7.25	+	.....	.....	.....	59...	7.4	+	21...	5.6	—
56.....	7.18	+	.....	.....	.....	63...	7.4	+	22...	5.6	—
90.....	7.06	+	.....	.....	.....	74...	7.4	+	23...	5.6	—
42.....	7.05	+	.....	.....	.....	81...	7.4	+	24...	5.6	—
70.....	6.93	+	.....	.....	.....	57...	7.3	+	30...	5.6	—
88.....	6.88	+	.....	.....	.....	90...	7.3	+	38...	5.6	—
34.....	6.72	—	.....	.....	.....	5 <sup>a</sup> ...	7.1	+	69...	5.6	—
40.....	6.72	+	.....	.....	.....	25...	7.0	+	71...	5.6	+
72.....	6.72	+	.....	.....	.....	40...	7.0	+	77...	5.6	—
33.....	6.56	—	.....	.....	.....	72...	7.0	+	9 <sup>a</sup> ...	5.5	—
48.....	6.52	+	.....	.....	.....	1 <sup>a</sup> ...	6.9	+	17 <sup>a</sup> ...	5.5	—
25.....	6.45	+	.....	.....	.....	4 <sup>a</sup> ...	6.9	+	49...	5.5	—
64.....	6.35	+	.....	.....	.....	88...	6.9	+	58...	5.5	—
54.....	6.18	+	.....	.....	.....	34...	6.8	—	61...	5.5	—
26.....	6.13	+	.....	.....	.....	70...	6.8	+	67...	5.5	—
36.....	6.12	+	.....	.....	.....	33...	6.7	—	76...	5.5	—
37.....	6.07	—	.....	.....	.....	26...	6.6	+	2 <sup>a</sup> ...	5.4	—
39.....	6.05	+	.....	.....	.....	48...	6.4	+	20...	5.4	—
68.....	6.05	+	.....	.....	.....	28...	6.2	+	51...	5.3	—

<sup>a</sup> P<sub>H</sub> not determined electrometrically.

The following summary of Tables V and VI needs little explanation.

*Summary of Tables V and VI*

Number of soils examined . . . . .	90
Number of soils containing Azotobacter . . . . .	51
Number of soils not containing Azotobacter . . . . .	39
Average mgm. nitrogen fixed, 84 soils . . . . .	6.25
Average mgm. nitrogen fixed, 45 soils containing Azotobacter . . . . .	8.32
Average mgm. nitrogen fixed, 39 soils not containing Azotobacter . . . . .	3.86
Number of soils electrometric $P_H$ 6.0 or above . . . . .	51
Number of soils electrometric $P_H$ below 6.0 . . . . .	39
Number of soils colorimetric $P_H$ 6.0 or above . . . . .	53
Number of soils colorimetric $P_H$ below 6.0 . . . . .	37
Number of soils electrometric $P_H$ 6.0 or above containing Azotobacter . . . . .	47
Number of soils electrometric $P_H$ 6.0 or above not containing Azotobacter . . . . .	4
Number of soils elcterometric $P_H$ below 6.0 containing Azotobacter . . . . .	4
Number of soils electrometric $P_H$ below 6.0 not containing Azotobacter . . . . .	35
Number of soils colorimetric $P_H$ 6.0 or above containing Azotobacter . . . . .	50
Number of soils colorimetric $P_H$ 6.0 or above not containing Azotobacter . . . . .	3
Number of soils colorimetric $P_H$ below 6.0 containing Azotobacter . . . . .	1
Number of soils colorimetric $P_H$ below 6.0 not containing Azotobacter . . . . .	36
Average electrometric $P_H$ , 71 soils . . . . .	6.57
Average colorimetric $P_H$ , 90 soils . . . . .	6.50
Average electrometric $P_H$ , 46 soils containing Azotobacter . . . . .	7.12
Average electrometric $P_H$ , 25 soils not containing Azotobacter . . . . .	5.57
Average colorimetric $P_H$ , 51 soils containing Azotobacter . . . . .	7.12
Average colorimetric $P_H$ , 39 soils not containing Azotobacter . . . . .	5.70
Association coefficient based on electrometric $P_H$ determinations . . . . .	0.981
Association coefficient based on colorimetric $P_H$ determinations . . . . .	0.997

It will be observed that the number of soils more alkaline than  $P_H$  6.0 by either method and not containing Azotobacter and also the number more acid than  $P_H$  6.0 containing Azotobacter are few. There were only four soils by the electrometric and three by the colorimetric method with a  $P_H$  of 6.0 or above that did not contain Azotobacter. One of the electrometric  $P_H$  6.0 or above soils (No. 46) not containing Azotobacter was more acid than  $P_H$  6.0 colorimetrically, while soil No. 37 was only slightly more alkaline than  $P_H$  6.0 by both methods. There were four soils with an electrometric and one with a colorimetric reaction more acid than  $P_H$  6.0 recorded as containing Azotobacter. Three of these gave a reaction less acid than  $P_H$  6.0 by one of the methods and the remaining samples did not give a typical Azotobacter growth.

SERIES II

In Table VII are recorded the data collected from the examination of the soils of Series II. Many of these soils were collected near the point where certain soils of Series I were taken and may thus be regarded as controls on the earlier examinations. In Table XIII are recorded in parallel columns the data secured from the two examinations. It will be noted that the data are quite similar even though two years intervened between the two analyses. Part of the data of Table VII are rearranged in Table VIII to show the possible association existing between the presence of Azotobacter and the hydrogen-ion concentration of the soil solution.



TABLE VII.—Type of growth, nitrogen fixed, and reaction of soils of Series II

Soil No.	Duplicate soil.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P <sub>n</sub> colorimetric.	P <sub>n</sub> electrometric.
				Mgm.			
101..	1	Typical Azotobacter.	Typical Azotobacter.	10.7	+	6.7	6.71
102..	2	None .....	No Azotobacter.....	6.3	—	5.0	3.78
103..	3	do .....	do .....	5.4	—	5.4	5.27
104..	4	Typical Azotobacter.	Typical Azotobacter.	10.5	+	7.1	.....
105..	5	do .....	do .....	11.8	+	7.2	7.08
106..	6	None .....	No Azotobacter.....	6.1	—	5.7	5.25
107..	31	Typical Azotobacter.	Typical Azotobacter.	10.2	+	7.4	7.70
108..	32	None .....	Azotobacter present.	8.5	+	5.6	5.48
109..	33	Typical Azotobacter.	Typical Azotobacter.	9.5	+	6.6	6.47
110..	34	do .....	do .....	7.9	+	7.4	7.61
111..	35	do .....	do .....	9.1	+	6.6	6.39
112..		Nontypical Azotobacter.	Azotobacter present.	.....	+	5.8	5.48
113..	36	Typical Azotobacter.	Typical Azotobacter.	8.9	+	5.6	5.85
114..	37	Nontypical Azotobacter.	do .....	8.9	+	5.8	5.59
115..	38	Typical Azotobacter.	do .....	8.6	+	5.4	5.51
116..	39	do .....	do .....	10.0	+	6.0	5.90
117..		do .....	do .....	10.3	+	6.6	6.56
118..		do .....	do .....	8.9	+	7.5	7.28
119..	40	do .....	do .....	9.8	+	7.1	7.00
120..	61	Nontypical Azotobacter.	Azotobacter present.	6.0	+	5.8	5.68
121..	66	None .....	No Azotobacter.....	7.6	—	5.7	5.24
122..	64	Nontypical Azotobacter. <sup>a</sup>	Azotobacter present.	8.6	+	5.8	5.51
123..	65	Typical Azotobacter.	Typical Azotobacter.	8.8	+	5.9	5.64
124..	63	do .....	do .....	9.0	+	6.9	7.06
125..		None .....	No Azotobacter.....	4.7	—	5.5	5.15
126..	49	do .....	do .....	8.0	—	5.5	5.19
127..	50	do .....	do .....	6.6	—	5.5	5.10
128..	51	do .....	do .....	5.1	—	5.9	5.78
129..	52	Typical Azotobacter.	Typical Azotobacter.	10.8	+	7.6	7.30
130..	53	do .....	do .....	7.3	+	7.4	7.52
131..	54	None .....	No Azotobacter.....	7.7	—	5.8	5.47
132..		Nontypical Azotobacter.	Typical Azotobacter.	9.3	+	5.9	5.54
133..	15	Typical Azotobacter.	do .....	13.5	+	7.3	7.45
134..	77	None .....	No Azotobacter.....	7.2	—	5.4	4.88
135..		Typical Azotobacter	Typical Azotobacter.	11.7	+	7.2	7.20
136..	16	None .....	Azotobacter present.	10.5	+	5.4	4.88
137..	17	do .....	No Azotobacter.....	4.3	—	5.8	5.58
138..	18	do .....	Azotobacter (?) .....	3.0	—	5.4	5.05
139..		do .....	do (?) .....	8.9	+	6.2	5.90
140..		Typical Azotobacter.	Typical Azotobacter.	7.3	+	7.0	7.30
141..		do .....	do .....	7.9	+	7.2	7.48
142..	82	do .....	do .....	9.7	+	7.8	7.59
143..	79	do .....	do .....	7.0	+	8.1	8.77
144..	80	do .....	do .....	8.8	+	7.3	7.72
145..	83	do .....	do .....	7.5	+	7.2	7.94
146..		do .....	do .....	8.1	+	6.9	7.16
147..		do .....	do .....	8.8	+	6.8	7.06
148..		do .....	do .....	10.3	+	7.3	7.69
149..		do .....	do .....	8.3	+	7.1	7.62
150..		do .....	do .....	6.4	+	7.3	7.65
151..		do .....	do .....	12.3	+	6.0	6.25
152..		do .....	do .....	0.4	+	6.0	5.06

<sup>a</sup> One sample.

TABLE VII.—*Type of growth, nitrogen fixed, and reaction of soils of Series II—Continued*

Soil No.	Duplicate soil.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P <sub>H</sub> colorimetric.	P <sub>H</sub> electrometric.
153.	.....	Nontypical Azotobacter.	Azotobacter present.	Mom. 2.8	—	5.6	5.59
154.	.....	Typical Azotobacter	Typical Azotobacter.	8.3	+	6.0	6.17
155.	.....	do.	do.	9.7	+	6.1	6.34
156.	.....	None	No Azotobacter.	9.6	—	5.5	5.61
157.	.....	Typical Azotobacter	Typical Azotobacter.	8.0	+	6.0	6.27
158.	.....	Nontypical Azotobacter.	Azotobacter present.	8.1	+	6.2	6.51
159.	.....	Typical Azotobacter	Typical Azotobacter.	8.3	+	7.8	7.77
160.	.....	do.	do.	10.1	+	6.2	6.89

TABLE VIII.—*Correlation between reaction and presence of Azotobacter in soils of Series II*

P <sub>H</sub> determined electrometrically.						P <sub>H</sub> determined colorimetrically.					
Soil No.	P <sub>H</sub> .	Azotobacter.	Soil No.	P <sub>H</sub> .	Azotobacter.	Soil No.	P <sub>H</sub> .	Azotobacter.	Soil No.	P <sub>H</sub> .	Azotobacter.
143.	8.77	+	151.	6.25	+	143.	8.1	+	155.	6.1	+
145.	7.94	+	154.	6.17	+	142.	7.8	+	116.	6.0	+
159.	7.77	+	152.	5.96	+	159.	7.8	+	151.	6.0	+
144.	7.72	+	139.	5.90	+	129.	7.6	+	152.	6.0	+
107.	7.70	+	116.	5.90	+	118.	7.5	+	154.	6.0	+
148.	7.69	+	113.	5.85	+	107.	7.4	+	157.	6.0	+
150.	7.65	+	128.	5.78	—	110.	7.4	+	123.	5.9	+
149.	7.62	+	120.	5.68	+	130.	7.4	+	128.	5.9	—
110.	7.61	+	123.	5.64	+	133.	7.3	+	132.	5.9	+
142.	7.59	+	156.	5.61	—	144.	7.3	+	112.	5.8	+
130.	7.52	+	153.	5.59	—	148.	7.3	+	114.	5.8	+
141.	7.48	+	114.	5.59	+	150.	7.3	+	120.	5.8	+
133.	7.45	+	137.	5.58	—	105.	7.2	+	122.	5.8	+
120.	7.30	+	132.	5.54	+	135.	7.2	+	131.	5.8	—
140.	7.30	+	122.	5.51	+	141.	7.2	+	137.	5.8	—
118.	7.28	+	115.	5.51	+	145.	7.2	+	106.	5.7	—
135.	7.20	+	112.	5.48	+	104 <sup>a</sup> .	7.1	+	121.	5.7	—
146.	7.16	+	108.	5.48	+	119.	7.1	+	108.	5.6	+
105.	7.08	+	131.	5.47	—	149.	7.1	+	113.	5.6	+
124.	7.06	+	103.	5.27	—	140.	7.0	+	153.	5.6	—
147.	7.06	+	106.	5.25	—	124.	6.9	+	125.	5.5	—
119.	7.00	+	121.	5.24	—	146.	6.9	+	126.	5.5	—
160.	6.89	+	126.	5.19	—	147.	6.8	+	127.	5.5	—
101.	6.71	+	125.	5.15	—	101.	6.7	+	156.	5.5	—
117.	6.56	+	127.	5.10	—	109.	6.6	+	103.	5.4	—
158.	6.51	+	138.	5.05	—	111.	6.6	+	115.	5.4	+
109.	6.47	+	134.	4.88	—	117.	6.6	+	134.	5.4	—
111.	6.39	+	136.	4.88	+	139.	6.2	+	136.	5.4	+
155.	6.34	+	102.	3.78	—	158.	6.2	+	138.	5.4	—
157.	6.27	+	.....	.....	.....	160.	6.2	+	102.	5.0	—

<sup>a</sup> P<sub>H</sub> not determined electrometrically.

In the following summary it may be observed that there were no soils more alkaline than  $P_H$  6.0 that did not contain *Azotobacter*. There were 13 soils with an electrometric and 10 with a colorimetric  $P_H$  below 6.0 that were recorded as containing *Azotobacter*. Only 5 of these produced typical *Azotobacter* film and 10 of the 13 gave a  $P_H$  of 5.8 or above by one of the methods. It would appear from the data of this series of soils that  $P_H$  5.8 was nearer the absolute limiting hydrogen-ion concentration than  $P_H$  6.0.

*Summary of Tables VII and VIII*

Number of soils examined.....	60
Number of soils containing <i>Azotobacter</i> .....	46
Number of soils not containing <i>Azotobacter</i> .....	14
Average mgm. nitrogen fixed, 59 soils.....	8.41
Average mgm. nitrogen fixed, 45 soils containing <i>Azotobacter</i> .....	9.16
Average mgm. nitrogen fixed, 14 soils not containing <i>Azotobacter</i> .....	6.03
Number of soils electrometric $P_H$ 6.0 or above.....	33
Number of soils electrometric $P_H$ below 6.0.....	27
Number of soils colorimetric $P_H$ 6.0 or above.....	36
Number of soils colorimetric $P_H$ below 6.0.....	24
Number of soils electrometric $P_H$ 6.0 or above containing <i>Azotobacter</i> .....	33
Number of soils electrometric $P_H$ 6.0 or above not containing <i>Azotobacter</i> .....	0
Number of soils electrometric $P_H$ below 6.0 containing <i>Azotobacter</i> .....	13
Number of soils electrometric $P_H$ below 6.0 not containing <i>Azotobacter</i> .....	14
Number of soils colorimetric $P_H$ 6.0 or above containing <i>Azotobacter</i> .....	36
Number of soils colorimetric $P_H$ 6.0 or above not containing <i>Azotobacter</i> .....	0
Number of soils colorimetric $P_H$ below 6.0 containing <i>Azotobacter</i> .....	10
Number of soils colorimetric $P_H$ below 6.0 not containing <i>Azotobacter</i> .....	14
Average electrometric $P_H$ , 59 soils.....	6.37
Average colorimetric $P_H$ , 60 soils.....	6.39
Average electrometric $P_H$ , 45 soils containing <i>Azotobacter</i> .....	6.73
Average electrometric $P_H$ , 14 soils not containing <i>Azotobacter</i> .....	5.21
Average colorimetric $P_H$ , 46 soils containing <i>Azotobacter</i> .....	6.65
Average colorimetric $P_H$ , 14 soils not containing <i>Azotobacter</i> .....	5.55
Association coefficient based on electrometric $P_H$ determinations.....	1.000
Association coefficient based on colorimetric $P_H$ determinations.....	1.000

The above summary with a few minor exceptions is very similar to that for Series I. The most marked difference is in the average nitrogen fixed by the soils not containing *Azotobacter*, this being 3.86 mgm. for Series I and 6.03 mgm. for Series II. This difference is due to the much higher fixation of nitrogen by soils not containing *Azotobacter* in media containing calcium carbonate than in the media without calcium carbonate. This fact has been emphasized in a former article (4). No calcium carbonate was added to the media employed in the examination of the soils of Series I, while an excess was added to the media in the examination of all subsequent soils.

**SERIES III**

The soils of Series III were secured through the county agents of Kansas and did not reach the laboratory in as fresh and possibly as uncontaminated condition as did those collected locally. Samples 138 to 180, inclusive, reached the laboratory while the writer was in the Army and remained several months before being examined. It was believed that the quantity of nitrogen fixed by these soils would not be comparable to that of fresh soils, hence no nitrogen determinations were made. Possibly these soils should be disregarded entirely. The data secured from the examination of the soils of this series are recorded in Tables IX and X, and summarized below.

TABLE IX.—Type of growth, nitrogen fixed, and reaction of soils of Series III

Soil No.	County.	Type of film.	Microscopic picture	Nitrogen fixed.	Azotobacter.	P <sub>H</sub> colorimetric.	P <sub>H</sub> electro-metric.
161.	Pottawattamie.	Typical Azotobacter.	Typical Azotobacter.	Mgm. 8.6	+	6.5	7.21
162.	do.	Nontypical film.	Azotobacter (?)...	7.1	+	5.7	6.02
163.	Cloud.	do.	do.	5.8	+	6.0	5.98
164.	do.	None	No Azotobacter.	5.0	—	5.7	5.41
165.	do.	do.	do.	4.4	—	5.6	5.54
166.	do.	do.	do.	7.0	—	5.5	5.71
167.	Wyandotte.	Nontypical.	Typical Azotobacter.	8.0	+	6.1	6.25
168.	Franklin.	None	No Azotobacter.	6.3	—	5.4	5.17
169.	do.	Nontypical.	Azotobacter present.	7.1	+	5.2	5.10
170.	Wilson	do.	do.	2.9	+	5.9	5.93
171.	do.	do.	do.	2.9	+	5.4	5.15
172.	do.	None	No Azotobacter.	5.1	—	5.3	5.02
173.	Pawnee.	Typical Azotobacter. <sup>a</sup>	Typical Azotobacter. <sup>a</sup>	8.2	+	6.0	6.08
174.	Franklin.	None	Azotobacter (?)...	5.4	—	5.8	5.85
175.	do.	do.	do.	3.0	—	5.4	5.29
176.	Anderson.	Typical Azotobacter.	Typical Azotobacter.	9.1	+	6.5	6.35
177.	do.	None	Azotobacter (?)...	7.2	—	5.6	5.37
178.	do.	do.	No Azotobacter.	9.6	—	5.6	5.30
179.	Nemaha.	do.	do.	4.7	—	5.8	5.76
180.	do.	do.	do.	1.8	—	5.6	5.47
181.	Marshall.	Nontypical	Azotobacter (?)...	7.9	+	5.9	6.10
182.	do.	None	No Azotobacter.	7.8	—	6.2	6.52
183.	do.	Typical Azotobacter.	Typical Azotobacter.	8.8	+	6.9	7.37
184.	do.	do.	do.	8.5	+	6.4	6.77
185.	Wyandotte.	None	No Azotobacter.	4.6	—	5.6	5.44
186.	do.	Typical Azotobacter.	Typical Azotobacter.	9.9	+	7.2	7.72
187.	do.	do.	do.	8.5	+	6.2	6.69
188.	Atchinson.	Nontypical	Azotobacter present.	7.4	+	5.6	5.52
189.	do.	None	No Azotobacter.	0.8	—	5.6	5.52
190.	do.	do.	Azotobacter present. <sup>a</sup>	5.5	—	5.6	5.34
191.	do.	do.	No Azotobacter.	5.3	—	5.7	5.32
192.	Wilson	do.	do.	7.2	—	5.6	5.22
193.	Jewell.	Nontypical.	Typical Azotobacter.	9.5	+	6.6	6.84
194.	do.	Typical Azotobacter.	do.	8.1	+	6.0	6.15
195.	Chase.	None	No Azotobacter.	7.7	—	5.8	5.69
196.	do.	do.	do.	6.6	—	5.4	4.90
197.	do.	Typical Azotobacter.	Typical Azotobacter.	6.9	+	6.6	7.57
198.	Lyon.	None	Azotobacter (?)...	4.6	—	5.9	5.49
199.	do.	Nontypical.	Typical Azotobacter.	5.1	+	5.9	6.07
200.	Osborne.	Typical Azotobacter.	do.	7.9	+	7.6	8.28
201.	do.	None	No Azotobacter.	6.1	—	6.1	5.98
202.	Kearney.	Typical Azotobacter.	Typical Azotobacter.	7.5	+	7.4	8.19
203.	do.	do.	do.	7.0	+	7.0	7.25

<sup>a</sup> One sample.

TABLE IX.—Type of growth, nitrogen fixed, and reaction of soils of Series III—Contd.

Soil No.	County.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P <sub>n</sub> colorimetric.	P <sub>n</sub> electro-metric.
				Mgm.			
204..	Washington.	None.....	No Azotobacter..	2.7	—	5.8	5.10
205..	Doniphan..	Typical Azotobacter.	Typical Azotobacter.	7.9	+	6.1	6.02
206..	do.....	do. <sup>a</sup> .....	do. <sup>a</sup> .....	5.4	—	5.7	5.32
207..	do.....	do.....	do.....	.....	+	7.1	7.94
208..	Rush.....	do.....	do.....	11.6	+	7.2	7.60
209..	Greenwood.	None.....	No Azotobacter..	0.3	—	5.7	5.53
210..	do.....	do.....	do.....	5.7	—	5.3	5.05
211..	do.....	do.....	do.....	4.0	—	5.3	5.10
212..	do.....	do.....	do.....	5.7	—	5.6	5.39
213..	Rawlins...	Typical Azotobacter.	Typical Azotobacter.	10.5	+	7.6	8.30
214..	do.....	do.....	do.....	10.0	+	7.3	7.98
215..	do.....	do.....	do.....	9.3	+	6.7	7.28
216..	do.....	do.....	do.....	9.6	+	6.9	7.40
217..	Greenwood.	do.....	do.....	11.1	+	5.6	5.15
218..	Jewell.....	do.....	do.....	10.6	+	6.6	7.28
219..	do.....	None.....	No Azotobacter..	6.0	—	6.6	7.25
220..	Pottawatomie.	Typical Azotobacter.	Typical Azotobacter.	11.2	+	6.4	6.34
221..	Ford.....	do. <sup>a</sup> .....	do. <sup>a</sup> .....	8.2	+	6.6	6.66
222..	do.....	do. <sup>a</sup> .....	do. <sup>a</sup> .....	11.8	+	6.5	6.69
223..	do.....	do.....	do.....	9.5	+	6.7	6.63
224..	do.....	do.....	do.....	9.3	+	6.9	7.52
225..	McPherson..	Nontypical.....	do.....	7.4	+	5.9	5.91
226..	do.....	Typical Azotobacter.	do.....	3.6	+	6.7	7.13
227..	do.....	None.....	No Azotobacter..	5.0	—	5.8	5.66
228..	do.....	do.....	do.....	5.1	—	5.6	5.20
229..	Burbon.....	Typical Azotobacter.	Typical Azotobacter.	10.2	+	7.1	7.60
230..	Osborne.....	do.....	do.....	8.9	+	6.6	6.98
231..	Kearney.....	Nontypical.....	do.....	7.0	+	7.4	8.25
232..	do.....	Typical Azotobacter.	do.....	10.1	+	7.4	8.31
233..	Greenwood.	Nontypical.....	do.....	7.0	+	7.0	7.47
234..	Meade.....	Typical Azotobacter.	do.....	8.1	+	7.4	8.15
235..	do.....	do.....	do.....	8.7	+	6.5	6.66
236..	Chautauqua.	None.....	No Azotobacter..	4.5	—	5.6	5.30
237..	do.....	do.....	Azotobacter (?)..	3.7	—	5.5	5.02
238..	Morris.....	do.....	No Azotobacter..	.....	—	5.7	5.54
239..	Pawnee.....	Typical Azotobacter.	Typical Azotobacter.	.....	+	6.1	6.08
240..	do.....	do.....	do.....	.....	+	6.6	7.03
241..	Meade.....	Nontypical.....	do.....	.....	+	6.4	6.18
242..	Elk.....	do. <sup>a</sup> .....	do. <sup>a</sup> .....	.....	—	5.9	5.51
243..	Meade.....	None.....	No Azotobacter..	.....	—	6.7	6.96
244..	Chautauqua.	do.....	Azotobacter (?)..	.....	—	5.1	5.22
245..	Rush.....	do.....	do.....	.....	—	6.1	6.54
246..	Douglas.....	do.....	No Azotobacter..	.....	—	5.8	5.90
247..	do.....	do.....	do.....	.....	—	5.5	5.37
248..	do.....	do.....	do.....	.....	—	5.7	5.47
249..	do.....	do.....	do.....	.....	—	5.4	5.12
250..	Coffey.....	Nontypical.....	Typical Azotobacter.	.....	+	5.9	6.00
251..	do.....	None.....	No Azotobacter..	.....	—	6.0	6.10

<sup>a</sup> One sample.

TABLE IX.—*Type of growth, nitrogen fixed, and reaction of soils of Series III—Contd.*

Soil No.	County.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P <sub>n</sub> colorimetric.	P <sub>n</sub> electrometric.
252..	Coffey .....	None .....	No Azotobacter .....	Mgm.	—	5.6	5.37
253..	Pawnee .....	Typical Azotobacter.	Typical Azotobacter.	.....	+	6.4	6.25
254..	Greenwood .....	None .....	No Azotobacter .....	.....	—	5.4	4.68
255..	Wichita .....	Typical Azotobacter.	Typical Azotobacter.	.....	+	7.2	7.79
256..	.....do.....	None .....	Azotobacter (?).....	.....	—	6.2	6.35
257..	.....do.....	Nontypical .....	Typical Azotobacter	.....	+	7.8	8.13
258..	.....do.....	Typical Azotobacter.	.....do.....	.....	+	7.6	8.26
259..	Logan .....	.....do.....	.....do.....	.....	+	6.5	6.49
260..	Thomas .....	None .....	Azotobacter (?).....	.....	—	7.0	7.48
261..	Pratt .....	.....do.....	No Azotobacter .....	.....	—	5.9	5.81
262..	.....do.....	.....do.....	.....do.....	.....	—	6.9	7.23
263..	Lyon .....	.....do.....	Azotobacter (?).....	.....	—	5.5	4.93
264..	Hodgeman .....	Typical Azotobacter.	Typical Azotobacter.	.....	+	7.4	7.52
271..	Woodson .....	None .....	No Azotobacter .....	.....	—	5.6	5.41
272..	Pratt .....	Typical Azotobacter.	Typical Azotobacter.	.....	+	7.4	7.72
273..	.....do.....	None .....	No Azotobacter .....	.....	—	6.5	6.42
274..	Finney .....	Nontypical .....	Typical Azotobacter.	.....	+	6.5	6.96
275..	.....do.....	Typical Azotobacter.	.....do.....	.....	+	7.4	8.06
276..	Jackson .....	None .....	No Azotobacter .....	.....	—	5.7	5.27
277..	.....do.....	.....do.....	.....do.....	.....	—	5.4	5.10
278..	Hodgeman .....	Nontypical .....	Azotobacter (?).....	.....	+	7.4	7.99
279..	.....do.....	None .....	No Azotobacter .....	.....	—	6.4	6.81
280..	.....do.....	Typical Azotobacter.	Typical Azotobacter.	.....	+	5.7	6.51

TABLE X.—Correlation between reaction and presence of *Azotobacter* in soils of Series III

PH determined electrometrically.						PH determined colorimetrically.					
Soil No.	PH	Azoto-bacter.	Soil No.	PH	Azoto-bacter.	Soil No.	PH	Azoto-bacter.	Soil No.	PH	Azoto-bacter.
232...	8.31	+	239...	6.08	+	257...	7.8	+	173...	6.0	+
213...	8.30	+	173...	6.08	+	200...	7.6	+	194...	6.0	+
200...	8.28	+	199...	6.07	+	213...	7.6	+	251...	6.0	—
258...	8.26	+	162...	6.02	+	258...	7.6	+	170...	5.9	+
231...	8.25	+	205...	6.02	+	202...	7.4	+	181...	5.9	+
202...	8.19	+	250...	6.00	+	231...	7.4	+	198...	5.9	—
234...	8.15	+	201...	5.98	—	232...	7.4	+	199...	5.9	+
257...	8.13	+	163...	5.98	+	234...	7.4	+	225...	5.9	+
275...	8.06	+	170...	5.93	+	264...	7.4	+	242...	5.9	—
278...	7.99	+	225...	5.91	+	272...	7.4	+	250...	5.9	+
214...	7.98	+	246...	5.90	—	275...	7.4	+	261...	5.9	—
207...	7.94	+	174...	5.85	—	278...	7.4	+	174...	5.8	—
255...	7.79	+	261...	5.81	—	214...	7.3	+	179...	5.8	—
272...	7.72	+	195...	5.80	—	186...	7.2	+	195...	5.8	—
186...	7.72	+	179...	5.76	—	208...	7.2	+	204...	5.8	—
208...	7.60	+	166...	5.71	—	255...	7.2	+	227...	5.8	—
229...	7.60	+	227...	5.66	—	207...	7.1	+	246...	5.8	—
197...	7.57	+	238...	5.54	—	229...	7.1	+	162...	5.7	+
264...	7.52	+	165...	5.54	—	203...	7.0	+	164...	5.7	—
224...	7.52	+	209...	5.53	—	233...	7.0	+	191...	5.7	—
260...	7.48	—	188...	5.52	+	260...	7.0	—	206...	5.7	—
233...	7.47	+	189...	5.52	—	183...	6.9	+	209...	5.7	—
216...	7.40	+	242...	5.51	—	216...	6.9	+	238...	5.7	—
183...	7.37	+	198...	5.49	—	224...	6.9	+	248...	5.7	—
218...	7.28	+	248...	5.47	—	262...	6.9	—	276...	5.7	—
215...	7.28	+	180...	5.47	—	215...	6.7	+	280...	5.7	+
219...	7.25	—	185...	5.44	—	223...	6.7	+	165...	5.6	—
203...	7.25	+	164...	5.41	—	226...	6.7	+	177...	5.6	—
262...	7.23	—	271...	5.41	—	243...	6.7	—	178...	5.6	—
161...	7.21	+	212...	5.39	—	193...	6.6	+	180...	5.6	—
226...	7.13	+	177...	5.37	—	197...	6.6	+	185...	5.6	—
240...	7.03	+	252...	5.37	—	218...	6.6	+	188...	5.6	+
230...	6.98	+	247...	5.37	—	219...	6.6	—	189...	5.6	—
243...	6.96	—	190...	5.34	—	221...	6.6	+	190...	5.6	—
274...	6.96	+	206...	5.32	—	230...	6.6	+	192...	5.6	—
182...	6.90	—	191...	5.32	—	240...	6.6	+	212...	5.6	—
193...	6.84	+	236...	5.30	—	161...	6.5	+	217...	5.6	+
279...	6.81	—	178...	5.30	—	176...	6.5	+	228...	5.6	—
184...	6.77	+	175...	5.29	—	222...	6.5	+	236...	5.6	—
222...	6.69	+	276...	5.27	—	235...	6.5	+	252...	5.6	—
187...	6.69	+	192...	5.22	—	259...	6.5	+	271...	5.6	—
235...	6.66	+	244...	5.22	—	273...	6.5	—	166...	5.5	—
221...	6.66	+	228...	5.20	—	274...	6.5	+	237...	5.5	—
223...	6.63	+	168...	5.17	—	184...	6.4	+	263...	5.5	—
245...	6.54	—	217...	5.15	+	220...	6.4	+	247...	5.5	—
280...	6.51	+	171...	5.15	+	241...	6.4	+	168...	5.4	—
259...	6.49	+	249...	5.12	—	253...	6.4	+	171...	5.4	+
273...	6.42	—	277...	5.10	—	279...	6.4	—	175...	5.4	—
256...	6.35	—	204...	5.10	—	182...	6.2	—	196...	5.4	—
176...	6.35	+	211...	5.10	—	187...	6.2	+	249...	5.4	—
220...	6.34	+	169...	5.10	+	256...	6.2	—	254...	5.4	—
253...	6.25	+	210...	5.05	—	167...	6.1	+	277...	5.4	—
167...	6.25	+	237...	5.02	—	201...	6.1	—	172...	5.3	—
241...	6.18	+	172...	5.02	—	205...	6.1	+	210...	5.3	—
194...	6.15	+	263...	4.93	—	239...	6.1	+	211...	5.3	—
251...	6.10	—	196...	4.90	—	245...	6.1	—	169...	5.2	+
181...	6.10	+	254...	4.68	—	163...	6.0	+	244...	5.1	—

*Summary of Tables IX and X*

Number of soils examined . . . . .	114
Number of soils containing Azotobacter . . . . .	60
Number of soils not containing Azotobacter . . . . .	54
Average mgm. nitrogen fixed, 76 soils . . . . .	6.95
Average mgm. nitrogen fixed, 44 soils containing Azotobacter . . . . .	8.28
Average mgm. nitrogen fixed, 32 soils not containing Azotobacter . . . . .	5.10
Number of soils electrometric $P_H$ 6.0 or above . . . . .	63
Number of soils electrometric $P_H$ below 6.0 . . . . .	51
Number of soils colorimetric $P_H$ 6.0 or above . . . . .	60
Number of soils colorimetric $P_H$ below 6.0 . . . . .	54
Number of soils electrometric $P_H$ 6.0 or above containing Azotobacter . . . . .	53
Number of soils electrometric $P_H$ 6.0 or above not containing Azotobacter . . . . .	10
Number of soils electrometric $P_H$ below 6.0 containing Azotobacter . . . . .	7
Number of soils electrometric $P_H$ below 6.0 not containing Azotobacter . . . . .	44
Number of soils colorimetric $P_H$ 6.0 or above containing Azotobacter . . . . .	49
Number of soils colorimetric $P_H$ 6.0 or above not containing Azotobacter . . . . .	11
Number of soils colorimetric $P_H$ below 6.0 containing Azotobacter . . . . .	11
Number of soils colorimetric $P_H$ below 6.0 not containing Azotobacter . . . . .	43
Average electrometric $P_H$ , 114 soils . . . . .	6.32
Average colorimetric $P_H$ , 114 soils . . . . .	6.21
Average electrometric $P_H$ , 60 soils containing Azotobacter . . . . .	6.94
Average electrometric $P_H$ , 54 soils not containing Azotobacter . . . . .	5.63
Average colorimetric $P_H$ , 60 soils containing Azotobacter . . . . .	6.62
Average colorimetric $P_H$ , 54 soils not containing Azotobacter . . . . .	5.77
Association coefficient based on electrometric $P_H$ determinations . . . . .	0.942
Association coefficient based on colorimetric $P_H$ determinations . . . . .	0.891

The data here summarized vary but slightly from those for the two former series. There were 10 soils with an electrometric and 11 with a colorimetric  $P_H$  of 6.0 or above that failed to show Azotobacter. Of these 11, 8 were among those that remained in the laboratory for several months before being examined. One of the remaining 3 reacted more acid and 1 only slightly less acid than  $P_H$  6.0 by one of the methods.

There were 7 soils that reacted electrometrically and 11 colorimetrically more acid than  $P_H$  6.0, recorded as containing Azotobacter. Six of these reacted less acid than  $P_H$  6.0 and 2 of the remaining less acid than  $P_H$  5.9 by one of the methods. Only 1 soil more acid than  $P_H$  5.9 produced a typical Azotobacter growth accompanied by good fixation of nitrogen.

## SERIES IV

In Table XI and XII data collected from the soils of Series IV are presented. These soils were collected in a large number of instances from experimental plots where the effect of lime upon acid soils was being studied. Such plots are usually located upon areas of acid soil. From observation such soils do not contain Azotobacter. The data here presented show that many of these limed soils should be, as far as reaction is concerned, favorable to the growth of Azotobacter, yet no Azotobacter were found. In other words, the addition of lime has changed the hydrogen-ion from an unfavorable to a favorable concentration for Azotobacter without apparently influencing the Azotobacter flora. There are many limed soils, however, to which the quantity of lime added apparently has not been sufficient to change appreciably the hydrogen-ion concentration.



TABLE X.—Correlation between reaction and presence of *Azotobacter* in soils of Series III

P <sub>H</sub> determined electrometrically.						P <sub>H</sub> determined colorimetrically.					
Soil No.	P <sub>H</sub>	Azoto- bacter.	Soil No.	P <sub>H</sub>	Azoto- bacter.	Soil No.	P <sub>H</sub>	Azoto- bacter.	Soil No.	P <sub>H</sub>	Azoto- bacter.
232...	8.31	+	239...	6.08	+	257...	7.8	+	173...	6.0	+
213...	8.30	+	173...	6.08	+	200...	7.6	+	194...	6.0	+
200...	8.28	+	199...	6.07	+	213...	7.6	+	251...	6.0	—
258...	8.26	+	162...	6.02	+	258...	7.6	+	170...	5.9	+
231...	8.25	+	205...	6.02	+	202...	7.4	+	181...	5.9	+
202...	8.19	+	250...	6.00	+	231...	7.4	+	198...	5.9	—
234...	8.15	+	201...	5.98	—	232...	7.4	+	199...	5.9	+
257...	8.13	+	163...	5.98	+	234...	7.4	+	225...	5.9	+
275...	8.06	+	170...	5.93	+	264...	7.4	+	242...	5.9	—
278...	7.99	+	225...	5.91	+	272...	7.4	+	250...	5.9	+
214...	7.98	+	246...	5.90	—	275...	7.4	+	261...	5.9	—
207...	7.94	+	174...	5.85	—	278...	7.4	+	174...	5.8	—
255...	7.79	+	261...	5.81	—	214...	7.3	+	179...	5.8	—
272...	7.72	+	195...	5.80	—	186...	7.2	+	195...	5.8	—
186...	7.72	+	179...	5.76	—	208...	7.2	+	204...	5.8	—
208...	7.60	+	166...	5.71	—	255...	7.2	+	227...	5.8	—
229...	7.60	+	227...	5.66	—	207...	7.1	+	246...	5.8	—
197...	7.57	+	238...	5.54	—	229...	7.1	+	162...	5.7	+
264...	7.52	+	165...	5.54	—	203...	7.0	+	164...	5.7	—
224...	7.52	+	209...	5.53	—	233...	7.0	+	191...	5.7	—
260...	7.48	—	188...	5.52	+	260...	7.0	—	206...	5.7	—
233...	7.47	+	189...	5.52	—	183...	6.9	+	209...	5.7	—
216...	7.40	+	242...	5.51	—	216...	6.9	+	238...	5.7	—
183...	7.37	+	198...	5.49	—	224...	6.9	+	248...	5.7	—
218...	7.28	+	248...	5.47	—	262...	6.9	—	276...	5.7	—
215...	7.28	+	180...	5.47	—	215...	6.7	+	280...	5.7	+
219...	7.25	—	185...	5.44	—	223...	6.7	+	165...	5.6	—
203...	7.25	+	164...	5.41	—	226...	6.7	+	177...	5.6	—
262...	7.23	—	271...	5.41	—	243...	6.7	—	178...	5.6	—
161...	7.21	+	212...	5.39	—	193...	6.6	+	180...	5.6	—
226...	7.13	+	177...	5.37	—	197...	6.6	+	185...	5.6	—
240...	7.03	+	252...	5.37	—	218...	6.6	+	188...	5.6	+
230...	6.98	+	247...	5.37	—	219...	6.6	—	189...	5.6	—
243...	6.96	—	190...	5.34	—	221...	6.6	+	190...	5.6	—
274...	6.96	+	206...	5.32	—	230...	6.6	+	192...	5.6	—
182...	6.90	—	191...	5.32	—	240...	6.6	+	212...	5.6	—
193...	6.84	+	236...	5.30	—	161...	6.5	+	217...	5.6	+
279...	6.81	—	178...	5.30	—	176...	6.5	+	228...	5.6	—
184...	6.77	+	175...	5.29	—	222...	6.5	+	236...	5.6	—
222...	6.69	+	276...	5.27	—	235...	6.5	+	252...	5.6	—
187...	6.69	+	192...	5.22	—	259...	6.5	+	271...	5.6	—
235...	6.66	+	244...	5.22	—	273...	6.5	—	166...	5.5	—
221...	6.66	+	228...	5.20	—	274...	6.5	+	237...	5.5	—
223...	6.63	+	168...	5.17	—	184...	6.4	+	263...	5.5	—
245...	6.54	—	217...	5.15	+	220...	6.4	+	247...	5.5	—
280...	6.51	+	171...	5.15	+	241...	6.4	+	168...	5.4	—
259...	6.49	+	249...	5.12	—	253...	6.4	+	171...	5.4	+
273...	6.42	—	277...	5.10	—	279...	6.4	—	175...	5.4	—
256...	6.35	—	204...	5.10	—	182...	6.2	—	196...	5.4	—
176...	6.35	+	211...	5.10	—	187...	6.2	+	249...	5.4	—
220...	6.34	+	169...	5.10	+	256...	6.2	—	254...	5.4	—
253...	6.25	+	210...	5.05	—	167...	6.1	+	277...	5.4	—
167...	6.25	+	237...	5.02	—	201...	6.1	—	172...	5.3	—
241...	6.18	+	172...	5.02	—	205...	6.1	+	210...	5.3	—
194...	6.15	+	263...	4.93	—	239...	6.1	+	211...	5.3	—
251...	6.10	—	196...	4.90	—	245...	6.1	—	169...	5.2	+
181...	6.10	+	254...	4.68	—	163...	6.0	+	244...	5.1	—

Summary of Tables IX and X

Number of soils examined	114
Number of soils containing Azotobacter	60
Number of soils not containing Azotobacter	54
Average mgm. nitrogen fixed, 76 soils	6.95
Average mgm. nitrogen fixed, 44 soils containing Azotobacter	8.28
Average mgm. nitrogen fixed, 32 soils not containing Azotobacter	5.10
Number of soils electrometric $P_H$ 6.0 or above	63
Number of soils electrometric $P_H$ below 6.0	51
Number of soils colorimetric $P_H$ 6.0 or above	60
Number of soils colorimetric $P_H$ below 6.0	54
Number of soils electrometric $P_H$ 6.0 or above containing Azotobacter	53
Number of soils electrometric $P_H$ 6.0 or above not containing Azotobacter	10
Number of soils electrometric $P_H$ below 6.0 containing Azotobacter	7
Number of soils electrometric $P_H$ below 6.0 not containing Azotobacter	44
Number of soils colorimetric $P_H$ 6.0 or above containing Azotobacter	49
Number of soils colorimetric $P_H$ 6.0 or above not containing Azotobacter	11
Number of soils colorimetric $P_H$ below 6.0 containing Azotobacter	11
Number of soils colorimetric $P_H$ below 6.0 not containing Azotobacter	43
Average electrometric $P_H$ , 114 soils	6.32
Average colorimetric $P_H$ , 114 soils	6.21
Average electrometric $P_H$ , 60 soils containing Azotobacter	6.94
Average electrometric $P_H$ , 54 soils not containing Azotobacter	5.63
Average colorimetric $P_H$ , 60 soils containing Azotobacter	6.62
Average colorimetric $P_H$ , 54 soils not containing Azotobacter	5.77
Association coefficient based on electrometric $P_H$ determinations	0.942
Association coefficient based on colorimetric $P_H$ determinations	0.891

The data here summarized vary but slightly from those for the two former series. There were 10 soils with an electrometric and 11 with a colorimetric  $P_H$  of 6.0 or above that failed to show Azotobacter. Of these 11, 8 were among those that remained in the laboratory for several months before being examined. One of the remaining 3 reacted more acid and 1 only slightly less acid than  $P_H$  6.0 by one of the methods.

There were 7 soils that reacted electrometrically and 11 colorimetrically more acid than  $P_H$  6.0, recorded as containing Azotobacter. Six of these reacted less acid than  $P_H$  6.0 and 2 of the remaining less acid than  $P_H$  5.9 by one of the methods. Only 1 soil more acid than  $P_H$  5.9 produced a typical Azotobacter growth accompanied by good fixation of nitrogen.

SERIES IV

In Table XI and XII data collected from the soils of Series IV are presented. These soils were collected in a large number of instances from experimental plots where the effect of lime upon acid soils was being studied. Such plots are usually located upon areas of acid soil. From observation such soils do not contain Azotobacter. The data here presented show that many of these limed soils should be, as far as reaction is concerned, favorable to the growth of Azotobacter, yet no Azotobacter were found. In other words, the addition of lime has changed the hydrogen-ion from an unfavorable to a favorable concentration for Azotobacter without apparently influencing the Azotobacter flora. There are many limed soils, however, to which the quantity of lime added apparently has not been sufficient to change appreciably the hydrogen-ion concentration.

TABLE XI.—Type of growth, nitrogen fixed, and reaction of soils from different States, Series IV

Soil No.	State.	Limed.	Type of film.	Microscopic picture.	Nitrogen fixed. <i>Mgm.</i>	Azotobacter.	P <sub>n</sub> colorimetric.	P <sub>n</sub> electrometric.
301	Michigan...	o	None.....	No Azotobacter.	4.2	—	5.9	5.76
302	....do....	o	Typical Azotobacter.	Typical Azotobacter.	7.1	+	5.6	6.64
303	....do....	o	None.....	No Azotobacter.	3.7	—	7.4	7.38
304	....do....	o	Typical Azotobacter.	Typical Azotobacter.	11.4	+	6.3	5.73
305	....do....	o	None.....	No Azotobacter.	4.8	—	5.9	5.69
306	California..	o	Nontypical...	Typical Azotobacter.	8.1	+	6.1	5.61
307	....do....	o	None.....	No Azotobacter.	6.5	—	5.9	5.49
308	....do....	o	....do....	Typical Azotobacter. <sup>a</sup>	5.6	—	6.3	5.81
309	....do....	o	Typical Azotobacter.	....do....	10.8	+	7.8	8.14
310	....do....	o	None.....	....do....	5.4	+	6.3	7.08
311	Ohio.....	+	....do....	No Azotobacter.	0.3	—	6.6	7.01
312	....do....	+	....do....	....do....	3.6	—	7.0	7.33
313	....do....	+	....do....	....do....	4.7	—	4.8	5.05
314	....do....	o	....do....	....do....	4.8	—	5.9	6.03
315	....do....	o	....do....	....do....	4.3	—	5.9	6.07
316	North Carolina.	o	....do....	....do....	4.5	—	4.6	5.00
317	....do....	+	....do....	....do....	6.0	—	6.4	6.78
318	....do....	+	....do....	....do....	5.0	—	5.9	6.01
319	New Jersey.	+	....do....	Azotobacter (?)	5.3	—	6.8	6.71
320	....do....	o	....do....	Azotobacter present.	4.8	—	4.9	4.80
321	....do....	+	....do....	Azotobacter (?)	6.0	—	6.4	6.52
322	....do....	+	....do....	Azotobacter present.	4.4	—	7.0	6.93
323	....do....	o	....do....	No Azotobacter.	4.4	—	4.9	4.90
324	Iowa.....	o	Typical Azotobacter.	Typical Azotobacter.	11.6	+	7.0	7.11
325	....do....	o	None.....	No Azotobacter.	4.9	—	4.9	4.78
326	....do....	o	....do....	....do....	4.8	—	4.8	4.83
327	....do....	o	Typical Azotobacter. <sup>a</sup>	Typical Azotobacter. <sup>a</sup>	6.5	+	4.9	4.93
328	....do....	o	None.....	No Azotobacter.	4.5	—	5.3	5.88
329	New York..	+	Typical Azotobacter.	Typical Azotobacter.	7.5	+	7.2	7.20
330	....do....	+	None.....	....do. <sup>a</sup> ....	5.5	+	7.0	6.84
331	....do....	+	Nontypical...	....do....	6.6	+	6.9	6.79
332	....do....	+	None.....	Azotobacter (?)	6.5	—	6.5	6.23
333	....do....	o	....do....	....do. (?)	5.7	—	5.2	5.47
334	Arkansas....	o	....do....	....do. (?)	7.8	+	7.0	6.96
335	....do....	o	....do....	....do. (?)	5.4	—	4.9	5.05
336	....do....	o	....do....	....do. (?)	5.0	—	5.5	5.58
337	Oregon....	o	....do....	No Azotobacter.	2.7	—	5.2	5.27
338	....do....	o	....do....	....do....	3.5	—	5.4	5.39
339	....do....	o	....do....	....do....	3.3	—	5.0	5.24
340	....do....	o	....do....	....do....	3.1	—	5.6	5.02

<sup>a</sup> One sample only.

TABLE XI.—*Type of growth, nitrogen fixed, and reaction of soils from different States, Series IV—Continued.*

Soil No.	State.	Limed.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P <sub>n</sub> colorimetric.	P <sub>n</sub> electrometric.
					<i>Mgm.</i>			
341	Oregon.....	o	None.....	Azotobacter...	3.5	—	5.5	5.66
342	Alabama.....	o	....do.....	....do.....	1.0	—	4.6	4.44
343	....do.....	o	....do.....	....do.....	1.6	—	5.9	5.83
344	....do.....	o	....do.....	....do.....	0.3	—	4.8	5.05
345	....do.....	+	....do.....	Azotobacter (?)	3.0	—	5.9	5.66
346	....do.....	+	....do.....	No Azotobacter.	1.7	—	5.9	5.51
347	Mississippi..	o	Typical Azotobacter.	Typical Azotobacter.	8.0	+	6.2	6.17
348	....do.....	o	None.....	Azotobacter (?)	2.5	—	4.6	4.21
349	....do.....	o	Typical Azotobacter.	Typical Azotobacter.	8.3	+	6.6	6.76
350	....do.....	o	....do.....	....do.....	8.6	+	6.2	6.20
351	....do.....	o	....do.....	....do.....	9.5	+	7.4	7.74
352	Illinois.....	+	....do.....	....do.....	.....	+	6.3	7.20
353	....do.....	o	None.....	No Azotobacter.	.....	—	4.8	5.00
354	....do.....	+	Typical Azotobacter.	Typical Azotobacter.	.....	+	5.8	6.51
355	....do.....	o	None.....	No Azotobacter.	.....	—	4.6	4.19
356	....do.....	o	....do.....	....do.....	.....	—	4.7	4.70
357	....do.....	+	....do.....	....do.....	.....	—	5.2	5.73
358	Indiana.....	o	....do.....	....do.....	4.0	—	4.3	3.80
359	....do.....	+	....do.....	....do.....	6.0	—	5.0	4.93
360	Georgia.....	+	....do.....	....do.....	5.8	—	6.6	6.69
361	....do.....	+	....do.....	....do.....	6.1	—	5.8	6.05
362	....do.....	o	....do.....	....do.....	6.6	—	5.0	5.24
363	....do.....	o	....do.....	....do.....	6.6	—	6.1	5.85
364	....do.....	o	....do.....	....do.....	5.8	—	4.8	4.76
365	Ohio.....	+	....do.....	....do.....	6.2	—	6.2	5.88
366	....do.....	+	Nontypical...	Typical Azotobacter.	6.8	+	6.8	6.84
367	....do.....	+	None.....	No Azotobacter.	5.1	—	6.2	6.18
368	....do.....	o	Nontypical...	Typical Azotobacter.	9.0	+	7.1	7.48
369	....do.....	o	....do.....	....do.....	10.4	+	6.7	7.05
370	....do.....	o	None.....	Azotobacter present.	9.6	+	7.0	7.45
371	New Jersey.	o	....do.....	No Azotobacter.	5.3	—	4.8	4.70
372	....do.....	+	....do.....	....do.....	5.1	—	5.7	5.37
373	....do.....	o	....do.....	....do.....	4.7	—	5.0	4.22
374	....do.....	+	....do.....	....do.....	5.3	—	6.5	6.44
375	....do.....	o	....do.....	....do.....	5.0	—	4.8	5.12
376	....do.....	+	....do.....	....do.....	5.4	—	6.8	6.86
377	North Carolina.	o	....do.....	....do.....	5.2	—	5.0	4.98
378	....do.....	o	Nontypical <sup>a</sup>	Typical Azotobacter. <sup>a</sup>	5.4	—	4.8	4.90
379	....do.....	o	None.....	No Azotobacter.	5.4	—	4.4	3.73
380	....do.....	o	....do.....	....do.....	5.2	—	5.0	4.93
381	....do.....	o	....do.....	....do.....	4.4	—	5.4	5.09
382	....do.....	o	....do.....	....do.....	5.8	—	5.0	4.63
383	Massachusetts.	+	Nontypical...	Azotobacter (?)	4.0	—	6.5	6.29
384	....do.....	+	....do.....	....do.(?).....	4.3	—	6.6	6.27

<sup>a</sup> One sample only.

TABLE XI—Type of growth, nitrogen fixed, and reaction of soils of different States, Series IV—Continued.

Soil No.	State.	Limed.	Type of film.	Microscopic picture.	Nitrogen fixed. Mgm.	Azotobacter.	P <sub>N</sub> colorimetric.	P <sub>N</sub> electrometric.
385	Massachusetts.	+	None . . . . .	No Azotobacter.	4.0	—	5.7	5.31
386	do . . . . .	+	do . . . . .	do . . . . .	4.1	—	5.6	5.24
387	do . . . . .	o	do . . . . .	do . . . . .	4.4	—	4.4	4.10
388	do . . . . .	o	do . . . . .	do . . . . .	3.5	—	4.6	4.01
389	Maryland . . . . .	o	do . . . . .	do . . . . .	6.4	—	4.8	4.70
390	do . . . . .	+	do . . . . .	do . . . . .	4.0	—	4.8	4.90
391	do . . . . .	+	do . . . . .	do . . . . .	4.2	—	6.6	6.77
392	do . . . . .	o	do . . . . .	do . . . . .	5.2	—	4.7	4.48
393	do . . . . .	o	do . . . . .	do . . . . .	4.5	—	4.7	4.51
394	do . . . . .	+	do . . . . .	do . . . . .	4.4	—	6.0	6.18
395	Rhode Island.	o	do . . . . .	do . . . . .	4.6	—	4.7	4.58
396	do . . . . .	+	do . . . . .	do . . . . .	2.7	—	5.6	5.64
397	do . . . . .	o	do . . . . .	do . . . . .	3.9	—	4.9	5.30
398	do . . . . .	+	do . . . . .	do . . . . .	4.6	—	6.6	6.77
399	do . . . . .	o	do . . . . .	do . . . . .	3.8	—	6.5	5.88
400	do . . . . .	+	do . . . . .	do . . . . .	3.9	—	4.6	4.31
401	Virginia . . . . .	+	Typical Azotobacter.	Typical Azotobacter.	6.0	+	5.6	6.15
402	do . . . . .	+	do . . . . .	do . . . . .	6.8	+	6.7	7.03
403	do . . . . .	+	do . . . . .	do . . . . .	6.8	+	7.0	7.30
404	do . . . . .	o	None . . . . .	No Azotobacter.	4.2	—	5.0	4.70
405	do . . . . .	o	do . . . . .	do . . . . .	5.8	—	4.9	4.75
406	do . . . . .	o	do . . . . .	do . . . . .	1.3	—	5.2	4.83
407	Kentucky . . . . .	o	do . . . . .	do . . . . .	6.6	—	4.9	4.82
408	do . . . . .	+	do . . . . .	do . . . . .	4.8	—	6.2	6.12
409	do . . . . .	o	do . . . . .	do . . . . .	4.2	—	5.8	5.19
410	do . . . . .	+	do . . . . .	do . . . . .	5.2	—	6.8	7.06
411	do . . . . .	o	do . . . . .	do . . . . .	7.0	—	4.8	4.82
412	do . . . . .	+	do . . . . .	do . . . . .	5.4	—	6.7	6.62
413	North Carolina.	o	do . . . . .	do . . . . .	3.9	—	6.0	6.28
414	do . . . . .	+	do . . . . .	do . . . . .	4.3	—	5.2	4.97
415	do . . . . .	o	do . . . . .	do . . . . .	3.9	—	4.9	4.81
416	do . . . . .	+	do . . . . .	do . . . . .	2.8	—	5.9	6.07
417	do . . . . .	o	do . . . . .	do . . . . .	3.5	—	4.8	4.66
418	Tennessee . . . . .	o	Nontypical . . . . .	Typical Azotobacter.	3.2	+	4.9	4.68
419	do . . . . .	+	do . . . . .	do . . . . .	2.5	+	5.5	5.69
420	do . . . . .	o	None . . . . .	Azotobacter (?)	1.0	—	4.8	4.56
421	do . . . . .	+	Nontypical . . . . .	Typical Azotobacter.	4.0	+	5.4	5.42
422	do . . . . .	o	do . . . . .	do . . . . .	3.2	+	4.8	4.53
423	do . . . . .	+	do . . . . .	do . . . . .	5.4	+	5.5	5.68
424	West Virginia.	o	None . . . . .	No Azotobacter.	4.8	—	4.6	4.09
425	do . . . . .	+	do . . . . .	do . . . . .	6.6	—	4.8	4.66
426	do . . . . .	o	do . . . . .	do . . . . .	4.2	—	4.6	4.02
427	do . . . . .	+	do . . . . .	do . . . . .	4.1	—	5.0	4.90
428	do . . . . .	o	do . . . . .	do . . . . .	3.3	—	4.6	3.99
429	do . . . . .	+	do . . . . .	do . . . . .	4.6	—	5.5	5.51
430	Indiana . . . . .	o	do . . . . .	do . . . . .	5.7	—	4.8	4.58
431	do . . . . .	+	Typical Azotobacter. <sup>a</sup>	Typical Azotobacter. <sup>a</sup>	8.3	+	5.6	5.03
432	New York . . . . .	+	do . . . . .	do . . . . .	6.9	+	6.7	7.30
433	do . . . . .	+	Nontypical <sup>a</sup> . . . . .	Azotobacter present.	5.1	—	6.6	6.78

<sup>a</sup> One sample only.

TABLE XI.—Type of growth, nitrogen fixed, and reaction of soils from different States, Series IV—Continued.

Soil No.	State.	Limed.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P <sub>H</sub> colorimetric.	P <sub>H</sub> electrometric.
434	New York..	+	Nontypical..	Typical Azotobacter.	Mgm. 6.1	+	6.2	6.84
435	.....do.....	+	None.....	Azotobacter (?)	7.3	—	5.7	6.23
436	.....do.....	o	.....do.....	No Azotobacter.	4.4	—	5.0	4.98
437	.....do.....	+	Typical Azotobacter.	Typical Azotobacter.	8.3	+	6.3	6.72
438	South Carolina.	o	Nontypical...	.....do.....	6.4	+	5.1	4.98
439	.....do.....	o	Typical Azotobacter.	.....do.....	8.9	+	7.6	8.14
440	.....do.....	+	.....do.....	.....do.....	6.8	+	6.8	7.84
441	.....do.....	o	Nontypical <sup>a</sup>	.....do.....	5.3	—	4.9	4.90
442	.....do.....	+	None.....	No Azotobacter.	4.2	—	4.9	5.15
443	.....do.....	+	.....do.....	.....do.....	4.4	—	5.2	5.34
444	Pennsylvania.	o	Typical Azotobacter.	Typical Azotobacter.	7.6	+	7.0	7.20
445	.....do.....	o	None.....	No Azotobacter.	5.6	—	5.2	5.59
446	.....do.....	o	.....do.....	Azotobacter (?)	6.3	—	4.8	5.07
447	.....do.....	+	Typical Azotobacter.	Typical Azotobacter.	8.0	+	7.4	8.41
448	.....do.....	+	.....do.....	.....do.....	8.1	+	8.2	8.25
449	.....do.....	o	None.....	No Azotobacter.	4.7	—	4.6	4.24
265	Missouri.....	o	.....do.....	.....do.....	5.0	—	4.7	4.36
266	.....do.....	o	.....do.....	.....do.....	4.5	—	5.2	4.97
267	.....do.....	+	Typical Azotobacter	Typical Azotobacter.	5.8	+	6.9	7.10
268	.....do.....	o	.....do.....	.....do.....	9.1	+	7.1	7.67
269	.....do.....	o	.....do.....	.....do.....	7.5	+	6.7	7.18

<sup>a</sup> One sample only.

TABLE XII.—Correlation between reaction and presence of *Azotobacter* in soils of Series IV

P <sub>H</sub> determined electrometrically.						P <sub>H</sub> determined colorimetrically.					
Soil No.	P <sub>H</sub> .	Azotobacter.	Soil No.	P <sub>H</sub> .	Azotobacter.	Soil No.	P <sub>H</sub> .	Azotobacter.	Soil No.	P <sub>H</sub> .	Azotobacter.
447...	8.41	+	445...	5.59	—	448...	8.2	+	401...	5.6	+
448...	8.25	+	336...	5.58	—	309...	7.8	+	431...	5.6	+
439...	8.14	+	429...	5.51	—	439...	7.6	+	429...	5.5	—
309...	8.14	+	346...	5.51	—	303...	7.4	—	336...	5.5	—
440...	7.84	+	307...	5.49	—	351...	7.4	+	341...	5.5	—
366...	7.84	+	333...	5.47	—	447...	7.4	+	419...	5.5	+
391...	7.77	—L <sup>a</sup>	421...	5.42	+	329...	7.2	+	423...	5.5	+
351...	7.74	+	338...	5.39	—	368...	7.1	+	338...	5.5	—
268...	7.67	+	372...	5.37	—	268...	7.1	+	381...	5.4	—
368...	7.48	+	443...	5.34	—	312...	7.0	—L	421...	5.4	+
370...	7.45	+	385...	5.31	—	322...	7.0	—L	328...	5.3	—
303...	7.38	—	397...	5.30	—	324...	7.0	+	443...	5.2	—
312...	7.33	—L	337...	5.27	—	330...	7.0	+	445...	5.2	—
403...	7.30	+	386...	5.24	—	334...	7.0	+	226...	5.2	—
432...	7.30	+	339...	5.24	—	370...	7.0	+	333...	5.2	—
352...	7.20	+	362...	5.24	—	403...	7.0	+	337...	5.2	—
444...	7.20	+	409...	5.19	—	444...	7.0	+	357...	5.2	—

<sup>a</sup> L=Limed soil.

TABLE XI.—Type of growth, nitrogen fixed, and reaction of soils from different States, Series IV—Continued.

P <sub>N</sub> determined electrometrically.						P <sub>N</sub> determined colorimetrically.					
Soil No.	P <sub>N</sub> .	Azoto-bacter.	Soil No.	P <sub>N</sub> .	Azoto-bacter.	Soil No.	P <sub>N</sub> .	Azoto-bacter.	Soil No.	P <sub>N</sub> .	Azoto-bacter.
329...	7.20	+	442...	5.15	—	331...	6.9	+	406...	5.2	—
269...	7.18	+	375...	5.12	—	267...	6.9	+	414...	5.2	—
324...	7.11	+	381...	5.09	—	319...	6.8	—L	438...	5.1	+
267...	7.10	+	344...	5.05	—	366...	6.8	+	436...	5.0	—
310...	7.08	+	335...	5.05	—	376...	6.8	—L	339...	5.0	—
410...	7.06	—L	313...	5.05	—	410...	6.8	—L	359...	5.0	—
369...	7.05	+	431...	5.03	+	440...	6.8	+	362...	5.0	—
402...	7.03	+	353...	5.00	—	369...	6.7	+	373...	5.0	—
311...	7.01	—L	316...	5.00	—	402...	6.7	+	377...	5.0	—
334...	6.96	+	438...	4.98	+	432...	6.7	+	380...	5.0	—
322...	6.93	—L	436...	4.98	—	269...	6.7	+	382...	5.0	—
376...	6.86	—L	377...	4.98	—	412...	6.7	—L	404...	5.0	—
434...	6.84	+	414...	4.97	—	311...	6.6	—L	427...	5.0	—
330...	6.84	+	266...	4.97	—	349...	6.6	+	320...	4.9	—
331...	6.79	+	446...	4.97	—	360...	6.6	—L	323...	4.9	—
317...	6.78	—L	327...	4.93	+	384...	6.6	—L	325...	4.9	—
433...	6.78	—L	380...	4.93	—	391...	6.6	—L	327...	4.9	+
398...	6.77	—L	359...	4.93	—	398...	6.6	—L	335...	4.9	—
349...	6.76	+	441...	4.90	—	433...	6.6	—L	397...	4.9	—
437...	6.72	+	427...	4.90	—	332...	6.5	—L	405...	4.9	—
319...	6.71	—L	323...	4.90	—	374...	6.5	—L	407...	4.9	—
360...	6.69	—L	378...	4.90	—	383...	6.5	—L	415...	4.9	—
302...	6.64	+	390...	4.90	—	399...	6.5	—L	418...	4.9	+
412...	6.62	—L	326...	4.83	—	317...	6.4	—L	441...	4.9	—
321...	6.52	—L	406...	4.83	—	321...	6.4	—L	442...	4.9	—
354...	6.51	+	407...	4.82	—	304...	6.3	+	313...	4.8	—
374...	6.44	—L	411...	4.82	—	308...	6.3	—	326...	4.8	—
383...	6.29	—L	415...	4.81	—	310...	6.3	+	344...	4.8	—
413...	6.28	—	320...	4.80	—	352...	6.3	+	353...	4.8	—
384...	6.27	—L	325...	4.78	—	437...	6.3	+	364...	4.8	—
435...	6.23	—L	364...	4.76	—	347...	6.2	+	371...	4.8	—
332...	6.23	—L	405...	4.75	—	330...	6.2	+	375...	4.8	—
350...	6.20	+	404...	4.70	—	365...	6.2	—L	378...	4.8	—
394...	6.18	—L	389...	4.70	—	367...	6.2	—L	446...	4.8	—
367...	6.18	—L	371...	4.70	—	408...	6.2	—L	430...	4.8	—
347...	6.17	+	356...	4.70	—	434...	6.2	+	425...	4.8	—
401...	6.15	+	418...	4.68	+	306...	6.1	+	422...	4.8	+
408...	6.12	—L	425...	4.66	—	363...	6.1	—	420...	4.8	—
416...	6.07	—L	417...	4.66	—	394...	6.0	—L	417...	4.8	—
315...	6.07	—	382...	4.63	—	413...	6.0	—	411...	4.8	—
361...	6.05	—L	420...	4.59	—	301...	5.9	—	390...	4.8	—
314...	6.03	—	395...	4.58	—	305...	5.9	—	389...	4.8	—
318...	6.01	—L	430...	4.58	—	307...	5.9	—	356...	4.7	—
340...	5.93	—	422...	4.53	+	314...	5.9	—	392...	4.7	—
328...	5.88	—	393...	4.51	—	315...	5.9	—	393...	4.7	—
365...	5.88	—	392...	4.48	—	318...	5.9	—	395...	4.7	—
399...	5.88	—	342...	4.44	—	343...	5.9	—	265...	4.7	—
363...	5.85	—	265...	4.36	—	345...	5.9	—	316...	4.6	—
343...	5.83	—	400...	4.31	—	346...	5.9	—	342...	4.6	—
308...	5.81	—	449...	4.24	—	416...	5.9	—	348...	4.6	—
301...	5.76	—	373...	4.22	—	354...	5.8	+	355...	4.6	—
357...	5.73	—	348...	4.21	—	361...	5.8	—	388...	4.6	—
304...	5.73	+	355...	4.19	—	409...	5.8	—	400...	4.6	—
305...	5.69	—	387...	4.10	—	435...	5.7	—	424...	4.6	—
419...	5.69	+	424...	4.09	—	385...	5.7	—	426...	4.6	—
423...	5.68	+	426...	4.02	—	372...	5.7	—	428...	4.6	—
341...	5.66	—	388...	4.01	—	302...	5.6	+	449...	4.6	—
345...	5.66	—	428...	3.99	—	340...	5.6	—	379...	4.4	—
396...	5.64	—	358...	3.80	—	386...	5.6	—	387...	4.4	—
306...	5.61	+	379...	3.73	—	396...	5.6	—	358...	4.3	—

It could not be expected that the mere correcting of the reaction of an acid, *Azotobacter*-free soil would bring about the establishment of an *Azotobacter* flora, unless accompanied by natural or artificial inoculation. Artificial inoculation with *Azotobacter* has been practiced to a very limited extent and it is probable that none of the soils examined have received such treatment. Natural inoculation would probably eventually occur in all cases. The rapidity with which it would take place would depend primarily upon the proximity of soils containing *Azotobacter* and upon the activity of the agencies operating in the transfer of soil from one to the other. Among these agencies would be wind, drainage, animals, insects, birds, etc., and the more or less artificial transfer upon agricultural tools and machinery. If soils containing *Azotobacter* are in close proximity, and the means of transfer are active, inoculation may occur very rapidly. For, in an effort to study the effect of inoculation upon adjacent plots, the writer found it impossible to prevent inoculation under certain conditions, while under other conditions such inoculation has not appeared during a period of three years. Some of the limed *Azotobacter*-free soils here recorded have been receiving lime for many years. As previously mentioned, many of the plots to which lime has been applied are located on rather large areas of strongly acid soils. Such plots may be some distance from areas that contain *Azotobacter* and, hence, chances for natural inoculation are poor. On the other hand, the writer repeatedly cultured the fertility plots of the Missouri Agricultural Experiment Station for *Azotobacter* during the years 1912-1914 with negative results. During 1914-15 certain of these plots received an addition of lime. When cultured for *Azotobacter* again in 1919 a vigorous flora was found to be present. At this last examination the reaction of the soil from a plot that had been limed was found to be neutral (soil No. 267, Table XI). Soil from adjacent unlimed plots was found to be strongly acid (soils Nos. 265 and 266, Table XI). In other words, decreasing the hydrogen-ion concentration of this soil to a point below  $1 \times 10^{-6}$  has resulted in the establishment of a vigorous *Azotobacter* flora in a comparatively short time. Other soils not far from these plots have been found to contain *Azotobacter* and it may be assumed that natural inoculation readily took place. It is not uncommon to find adjacent plots or areas of natural soil one of which contains *Azotobacter* while the other does not.

*Summary of Tables XI and XII*

Number of soils examined.....	154
Number of soils containing <i>Azotobacter</i> .....	42
Number of soils not containing <i>Azotobacter</i> .....	112
Average mgm. nitrogen fixed, 148 soils.....	5.30
Average mgm. nitrogen fixed, 40 soils containing <i>Azotobacter</i> .....	7.35
Average mgm. nitrogen fixed, 108 soils not containing <i>Azotobacter</i> .....	4.53
Number of soils electrometric $P_H$ 6.0 or above.....	60
Number of soils electrometric $P_H$ below 6.0.....	94
Number of soils colorimetric $P_H$ 6.0 or above.....	57
Number of soils colorimetric $P_H$ below 6.0.....	97
Number of soils electrometric $P_H$ 6.0 or above containing <i>Azotobacter</i> .....	32
Number of soils electrometric $P_H$ 6.0 or above not containing <i>Azotobacter</i> .....	28
Number of limed soils electrometric $P_H$ 6.0 or above not containing <i>Azotobacter</i> .....	24
Number of normal soils electrometric $P_H$ 6.0 or above not containing <i>Azotobacter</i> .....	4
Number of soils electrometric $P_H$ below 6.0 containing <i>Azotobacter</i> .....	10
Number of soils electrometric $P_H$ below 6.0 not containing <i>Azotobacter</i> .....	84



Number of soils colorimetric $P_H$ 6.0 or above containing Azotobacter.....	31
Number of soils colorimetric $P_H$ 6.0 or above not containing Azotobacter....	26
Number of limed soils colorimetric $P_H$ 6.0 or above not containing Azotobacter..	22
Number of normal soils colorimetric $P_H$ 6.0 or above not containing Azotobacter..	4
Number of soils colorimetric $P_H$ below 6.0 containing Azotobacter.....	11
Number of soils colorimetric $P_H$ below 6.0 not containing Azotobacter.....	86
Average electrometric $P_H$ , 154 soils.....	5.72
Average colorimetric $P_H$ , 154 soils.....	5.70
Average electrometric $P_H$ , 42 soils containing Azotobacter.....	6.70
Average electrometric $P_H$ , 112 soils not containing Azotobacter.....	5.35
Average colorimetric $P_H$ , 42 soils containing Azotobacter.....	6.46
Average colorimetric $P_H$ , 112 soils not containing Azotobacter.....	5.42
Association coefficient based on electrometric $P_H$ determinations.....	0.971
Association coefficient based on colorimetric $P_H$ determinations.....	0.968

Liming is an artificial method by which the reaction of soils can suddenly be changed from an unfavorable to a favorable condition for the growth of Azotobacter. Unless accompanied by inoculation with Azotobacter, soils that have thus been rendered favorable and do not contain Azotobacter should not be classed with soils that normally have a favorable reaction and do not contain Azotobacter. For these reasons the limed soils with reactions of  $P_H$  6.0 or above and not containing Azotobacter have been separated from the others and are not considered in the association coefficient calculations.

In Series IV there were 28 soils tested by the electrometric and 26 by the colorimetric method (32 different soils) with a reaction of  $P_H$  6.0 or above that did not contain Azotobacter. Twenty-four of the former and 22 of the latter are known to have been limed more or less recently. There were, therefore, only 6 unlimed soils with  $P_H$  6.0 or above by one of the methods that did not contain Azotobacter. Five of the 6 reacted more acid than  $P_H$  6.0 by one of the methods, leaving only 1 unlimed soil definitely more acid than  $P_H$  6.0 and not containing Azotobacter. It is probable that lime has been applied to some of the soils of which no record is available. In a few instances the data indicate that some unknown factor has been influencing the reaction. (Compare soils 413 with 414, 438 with 439, 399 with 400, and 363 with 364.) It is believed that the data on history were incorrectly recorded in a few instances. There were 10 soils that reacted electrometrically and 11 colorimetrically (13 different soils) more acid than  $P_H$  6.0 in which no Azotobacter were recorded. Five of these were less acid than  $P_H$  6.0 by one of the methods; not one of the remaining 8 had a typical Azotobacter growth accompanied by good fixation of nitrogen in both samples.

#### GENERAL SUMMARY AND DISCUSSION

Number of soils examined.....	418
Number of soils containing Azotobacter.....	199
Number of soils not containing Azotobacter.....	219
Average mgm. nitrogen fixed, 367 soils.....	6.36
Average mgm. nitrogen fixed, 174 soils containing Azotobacter.....	8.30
Average mgm. nitrogen fixed, 193 soils not containing Azotobacter.....	4.61
Number of soils electrometric $P_H$ 6.0 or above.....	207
Number of soils electrometric $P_H$ below 6.0.....	211
Number of soils colorimetric $P_H$ 6.0 or above.....	206
Number of soils colorimetric $P_H$ below 6.0.....	212
Number of soils electrometric $P_H$ 6.0 or above containing Azotobacter.....	165
Number of soils electrometric $P_H$ 6.0 or above not containing Azotobacter....	42
Number of limed soils electrometric $P_H$ 6.0 or above not containing Azotobacter..	24

Number of normal soils electrometric $P_H$ 6.0 or above not containing Azotobacter	18
Number of soils electrometric $P_H$ below 6.0 containing Azotobacter	34
Number of soils electrometric $P_H$ below 6.0 not containing Azotobacter	177
Number of soils colorimetric $P_H$ 6.0 or above containing Azotobacter	166
Number of soils colorimetric $P_H$ 6.0 or above not containing Azotobacter	40
Number of limed soils colorimetric $P_H$ 6.0 or above not containing Azotobacter	22
Number of normal soils colorimetric $P_H$ 6.0 or above not containing Azotobacter	18
Number of soils colorimetric $P_H$ below 6.0 containing Azotobacter	33
Number of soils colorimetric $P_H$ below 6.0 not containing Azotobacter	179
Average electrometric $P_H$ , 398 soils	6.09
Average colorimetric $P_H$ , 418 soils	6.11
Average electrometric $P_H$ , 193 soils containing Azotobacter	6.88
Average electrometric $P_H$ , 205 soils not containing Azotobacter	5.44
Average colorimetric $P_H$ , 199 soils containing Azotobacter	6.72
Average colorimetric $P_H$ , 210 soils not containing Azotobacter	5.56
Association coefficient based on electrometric $P_H$ determinations	0.959
Association coefficient based on colorimetric $P_H$ determinations	0.961

In this summary there are a few points to which the writer wishes to call special attention. Little is known of the practical significance of Azotobacter in the nitrogen economy of soils. After carefully reviewing all the available literature and data on the subject Greaves (8) says:

In conclusion, it may be stated that, although the part played by Azotobacter in maintaining the nitrogen of the soil has not been definitely measured, it is nevertheless an important factor. It is, therefore, conservative to state that these organisms under favorable conditions add from 15 to 40 pounds of available nitrogen to each acre of soil yearly.

If these figures are a conservative estimate of the quantity of nitrogen fixed by these organisms, the presence or absence of such a flora in soils is of enormous economic importance. Yet, more than half the soils examined during the past few years apparently do not contain Azotobacter. There are organisms present in practically all soils capable of fixing some nitrogen under laboratory conditions. The quantity of nitrogen fixed under the conditions of these experiments is only approximately half as great in the absence of Azotobacter as when they are present. It is believed that under actual soil conditions the differences are much more marked than these figures indicate. If this important group of organisms is absent from such a large per cent of soils, what are the factors controlling their presence and absence and how can the unfavorable conditions be remedied?

In a former publication (2) an apparent close correlation between the absolute reaction of the soil solution and the presence of Azotobacter in soils was shown. The present data demonstrate the existence of such a correlation. By using Yule's association formula the very high coefficient of 0.96 is obtained, indicating an unmistakable correlation. Very few soils in which the hydrogen-ion concentrations exceed  $1 \times 10^{-8}$  contain Azotobacter, while practically 11 soils with a hydrogen-ion concentration less than  $1 \times 10^{-8}$  contain an active Azotobacter flora. The per cent of soils of different hydrogen-ion concentrations containing Azotobacter is found in Table XIV and shown graphically in figure 1.

TABLE XIII.—Comparison of the presence of *Azotobacter*, nitrogen fixed, and reaction of same soils collected 2 years apart

Soil No.		Azotobacter.		Nitrogen fixed.		Colorimetric P <sub>H</sub> .		Electrometric P <sub>H</sub> .	
1918	1920	1918	1920	1918	1920	1918	1920	1918	1920
				Mgm.	Mgm.				
1.....	101	+	+	10.3	10.7	6.9	6.7	.....	6.71
2.....	102	—	—	2.0	6.3	5.4	5.0	.....	3.78
3.....	103	—	—	3.0	5.4	5.6	5.4	.....	5.27
4.....	104	+	+	6.8	10.5	6.9	7.1	.....	.....
5.....	105	+	+	5.4	11.8	7.1	7.2	.....	7.08
6.....	106	—	—	4.4	6.1	5.7	5.7	.....	5.25
15.....	133	+	+	10.3	13.5	7.4	7.3	.....	7.45
16.....	136	—	+	4.0	10.5	5.6	5.4	.....	4.88
17.....	137	—	—	3.0	4.3	5.5	5.8	.....	5.58
18.....	138	—	—	3.7	3.0	5.6	5.4	.....	5.05
31.....	107	+	+	10.6	10.2	7.5	7.4	7.71	7.70
32.....	108	—	+	5.6	8.5	5.9	5.6	5.85	5.48
33.....	109	—	+	4.0	9.5	6.7	6.6	6.56	6.47
34.....	110	—	+	4.5	7.9	6.8	7.4	6.74	7.61
35.....	111	+	+	6.8	9.1	7.6	6.6	7.62	6.39
36.....	113	+	+	9.2	8.9	6.0	5.6	6.12	5.85
37.....	114	—	+	5.4	8.9	6.2	5.8	6.07	5.59
38.....	115	—	+	3.9	8.6	5.6	5.4	5.59	5.51
39.....	116	+	+	8.6	10.0	6.1	6.0	6.05	5.90
40.....	119	+	+	8.1	9.8	7.0	7.1	6.72	7.00
49.....	126	—	—	2.7	8.0	5.5	5.5	5.03	5.19
50.....	127	—	—	4.0	6.6	5.8	5.5	5.32	5.10
51.....	128	—	—	3.2	5.1	5.3	5.9	5.41	5.78
52.....	129	+	+	5.5	10.8	7.3	7.6	7.27	7.30
53.....	130	+	+	9.0	7.3	7.7	7.4	7.79	7.52
54.....	131	+	—	7.4	7.7	6.0	5.8	6.08	5.47
61.....	120	—	+	3.7	6.0	5.5	5.8	5.51	5.68
63.....	124	+	+	11.0	9.0	7.4	6.9	7.43	7.06
64.....	122	+	+	8.3	8.6	6.1	5.8	6.35	5.51
65.....	123	—	+	5.2	8.8	5.7	5.9	5.94	5.64
66.....	121	—	—	3.8	7.6	5.7	5.7	5.81	5.24
77.....	134	—	—	4.2	7.2	5.6	5.4	5.34	4.88
79.....	143	+	+	7.6	7.0	7.7	8.1	8.48	8.77
80.....	144	+	+	9.2	8.8	7.6	7.3	7.84	7.72
82.....	142	+	+	8.2	9.7	7.7	7.8	7.60	7.59
83.....	145	+	+	9.9	7.5	7.6	7.2	7.72	7.94

TABLE XIV.—The number and percentage of soils of various reactions containing *Azotobacter*

P <sub>H</sub> reaction.	Electrometric.					Colorimetric.				
	Number of soils.	Azotobacter.				Number of soils.	Azotobacter.			
		Present.		Absent.			Present.		Absent.	
		No.	Per cent.	No.	Per cent.		No.	Per cent.	No.	Per cent.
Above 7.50 .....	57	57	100	0	0	32	32	100	0	0
7.00 to 7.49 ....	49	45	92	4	8	60	58	97	2	3
6.50 to 6.99 ....	33	27	82	6	18	45	39	90	6	10
6.00 to 6.49 ....	38	30	80	8	20	47	37	80	10	20
5.50 to 5.99 ....	64	22	34	42	66	122	24	20	98	80
5.00 to 5.49 ....	75	7	9	68	91	43	6	14	37	86
4.50 to 4.99 ....	42	5	12	37	88	44	3	7	41	93
Below 4.50 .....	16	0	0	16	100	3	0	0	3	100
Total .....	374	193	...	181	...	396	199	...	197	...

The existence in soils of an excess of hydrogen over hydroxyl-ions is no longer questioned. In 75 per cent of the 418 soils tested an excess of hydrogen over hydroxyl-ions was found whether the concentration was measured electrometrically or colorimetrically. A large number of examples are on record showing the toxic effect of a high concentration of hydrogen-ions upon bacteria. The writer has recently shown (6) that pure cultures of *Azotobacter* will not grow in laboratory media with a hydrogen-ion concentration appreciably in excess of  $1 \times 10^{-6}$ .

It is quite generally accepted that soil acidity is indirectly responsible for certain plant root diseases, and it is possible that the influence of the absolute reaction of the soil solution upon *Azotobacter* here indicated is also indirect. However, since the same identical limiting hydrogen-ion concentration has been found with pure cultures, in laboratory media (6) as in soil solutions, it is believed that the recorded absence of this group

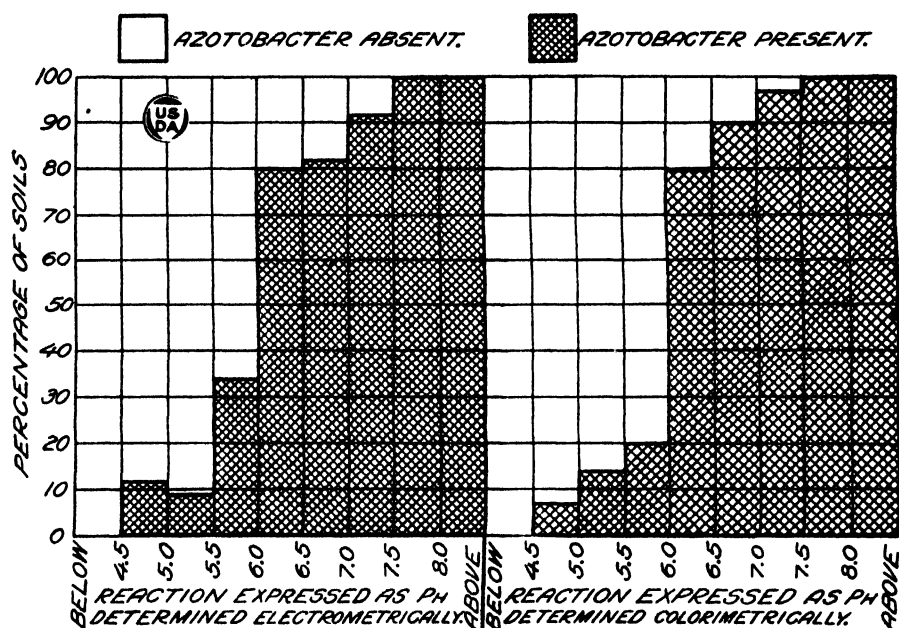


FIG. 1.—Percentage of soils of different reactions containing *Azotobacter*

of organisms in the soils examined is in most instances correct and is directly due to the toxic effect of the high concentration of hydrogen-ions existing in the soil solution. Furthermore, it is believed that the maximum hydrogen-ion concentration tolerated by this group of organisms is very near  $1 \times 10^{-6}$  in soils as well as in laboratory media. Whether or not the hydrogen-ions are the ultimate limiting agent, the fact has been clearly established that the two are definitely associated. It is also evident from the data accumulated in this laboratory and shown elsewhere (3) that the same factors that will raise the hydrogen-ion concentration of a soil solution appreciably above, or lower it below,  $1 \times 10^{-6}$  will also render the soil capable or incapable, as the case may be, of supporting an *Azotobacter* flora.

How can the unfavorable conditions for the existence and growth of *Azotobacter* in soils be corrected? Attention has been called to the fact that a reduction in the hydrogen-ion concentration is probably the

one essential condition to be fulfilled, in rendering a soil capable of supporting *Azotobacter*. This has been accomplished experimentally in a number of different ways. Perhaps the simplest and most economical one is the addition of sufficient lime to reduce the hydrogen-ion concentration to a point below  $1 \times 10^{-6}$ . Attention has also been called (3) to the ease with which this can be done in the laboratory. In a forthcoming publication it will be shown that the same is true under natural soil conditions. The most acid soil that has been encountered locally required approximately 18,000 pounds of calcium carbonate to reduce the acidity to below  $P_H$  6.0. Three years ago sufficient lime to accomplish this was added under natural conditions accompanied by inoculation both with soil containing *Azotobacter* and with cultures grown in the laboratory. These plots still contain a vigorous *Azotobacter* flora and will fix, in laboratory media, two to three times as much nitrogen as will adjacent, similarly inoculated, unlimed plots from which the *Azotobacter* disappeared within a few days following inoculation.

It is not the purpose of this paper to advocate the use of lime and *Azotobacter* inoculation solely as a means of aiding the maintenance of the nitrogen supply of soils. For, while it is believed that this factor alone would justify the expense, the economic phases of the proposition have not been sufficiently investigated.

Experiments are now under way that promise to solve this phase of the problem locally. However, it is generally agreed that permanent agriculture is impossible without a soil sufficiently supplied with lime. Nature has provided ample means for maintaining the nitrogen supply of native soils. The American farmer has so ignored nature's methods that many soils have become depleted of their nitrogen supply and are productive only when supplied artificially with nitrogen at an enormous expense. One of nature's methods is through the agency of different types of nitrogen-fixing bacteria. One essential requirement of these organisms is a low hydrogen-ion concentration in the soil. While the quantity of nitrogen fixed by these organisms might not alone justify the expense of maintaining a low degree of acidity, this factor, coupled with the other recognized physicochemical benefits to the soil and physiological benefits to the growing plants, would unquestionably justify such a practice. The possible influence of adequate liming upon the nitrogen economy of the soil is mentioned as an added stimulus to the use of lime.

Another point to be noted is the general similarity of results, whether based upon the electrometric or the colorimetric method of determining the hydrogen-ion concentration of the soil solution. The individual determinations frequently do not agree as closely as might be desired. Yet the conclusions, so far as this study is concerned, would be identical regardless of data upon which they were based. In a general way the farther removed from neutrality, the wider the variation between the two methods. The electrometric method usually recorded a higher or lower hydrogen-ion concentration, as the case might be, than the colorimetric method if the soil were appreciably acid or alkaline. It is believed, however, that the general agreement between the two methods is sufficiently close to justify the use of the much quicker and less expensive colorimetric method as an aid in the solution of many soil problems, particularly where the initial expense prohibits the purchase of the necessary expensive electrometric apparatus.

The possibility of a biological means of determining the lime requirements of soils is indicated by the data here presented. If a series of groups or of individual microorganisms with definite limiting hydrogen-ion concentrations could be isolated from soils it would only be necessary to determine the presence or absence of certain species in order to establish the absolute reaction of the soil solution in situ. Furthermore, it would be comparatively simple to ascertain how much lime to add in order to render the soil capable of supporting the various species. It has been possible to determine with an accuracy of a few hundred pounds the quantity of lime necessary to add to soils more acid than  $P_H$  6.0 in order to render them capable of supporting *Azotobacter*. It is believed that not until the plant physiologist has established the optimum and limiting hydrogen-ion concentrations of the soil solution for the various agricultural plants, and the bacteriologist has associated these limits with definite groups of microorganisms, will a true and accurate physiological basis for determining and correcting the reaction of soils be possible. *Azotobacter* can now be used as a biological means of separating soils into two groups, depending upon reaction; and the quantity of lime necessary to change the reaction of the more acid group into the less acid group can be definitely determined.

#### CONCLUSIONS

(1) Seventy-five per cent of the 418 soils examined were found to be acid. Fifty per cent were more acid than  $P_H$  6.0 and 50 per cent apparently did not contain *Azotobacter*.

(2) In general the electrometric and colorimetric methods of determining the hydrogen-ion concentration of the soil solution agreed.

(3) A definite and very close correlation has been established between the absolute reaction of the soil solution and the presence or absence of *Azotobacter* in the soil.

(4) Very few soils more acid than indicated by a hydrogen-ion concentration of  $1 \times 10^{-6}$  contain *Azotobacter*, while this group of organisms is in most instances present in soils with a lower hydrogen-ion concentration. Since a very similar limiting hydrogen-ion concentration has been found for pure cultures of *Azotobacter* in laboratory media, it is believed that the very close correlation existing between the reaction and the presence of *Azotobacter* indicates that the absolute reaction is of paramount importance, if not the actual limiting factor, in controlling the presence of this group of organisms in soils.

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# A STUDY OF FACTORS AFFECTING THE NITROGEN CONTENT OF WHEAT AND OF THE CHANGES THAT OCCUR DURING THE DEVELOPMENT OF WHEAT<sup>1</sup>

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## INTRODUCTION

The subject matter of this paper pertains to a study of the nitrogen content of wheat produced under various experimental conditions and a study of changes that occur during the development of wheat. The investigation had its inception in studies of nitrogen content of wheat by Thatcher (31, 32, 33).<sup>3</sup>

In the States of California, parts of Idaho, Montana, Oregon, Utah, and Washington, wheat, irrespective of variety, generally develops large, plump kernels of comparatively soft texture. The nitrogen or gluten content of these soft wheats is variable, with localized districts apparently more favorable to the production of wheat with high percentages of nitrogen or gluten than is observed in others. Farther east, in the States of North Dakota, South Dakota, Nebraska, Kansas, Minnesota, Missouri, and Iowa the wheat kernels, generally, are of smaller size. These smaller-sized kernels, however, may be plump yet the texture of the grain may be comparatively harder. The percentage of nitrogen or gluten in this harder type of wheat varies but, generally speaking, is comparatively higher than in the softer wheats.

The influence of exchange of seed, breeding new varieties, selections for nitrogen content, soil types, soil treatment, fertilizers, water, and temperature on the percentage of nitrogen in wheat have been studied by various investigators. From the results of investigation, it is held that the variation in composition of wheat is due, chiefly, to variation in environmental conditions, such as rain, sunshine, humidity of the atmosphere, methods of harvesting, methods of cultivations, and amount of manure. The effect of continuous cropping, rotation, the method and time of sowing seed, including the quantity and quality of the seed sown, have also been studied. With the exception of irrigation, the first three of these conditions are wholly uncontrollable; the remainder of them can be regulated, and it is to these that the investigational work has been and should be directed to determine whether or not the nitrogen content of wheat can be increased.

## HISTORICAL REVIEW

### EFFECT OF CLIMATE ON NITROGEN CONTENT OF WHEAT

Over 60 years ago, Lawes and Gilbert (7) concluded from their experiments that long periods of growth produced plump kernels of low nitrogen content. Later these men (8) stated that—

a season favorable for long and continuous growth after heading, produced well developed kernels and larger yields; the mature grain developed under the most favorable conditions contained a high amount of starch and a low amount of potash, phosphoric acid, and nitrogen.

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<sup>3</sup> Reference is made by number (*italic*) to "Literature used," p. 951-953.



Richardson (18) and Brewer (3) concurred that climate exerted the greatest influence. According to Wiley (39, *p.* 244; 40) a cool climate and short periods of growth are favorable for high protein, while a warm climate and long periods of growth are favorable for low protein content. Jensen (6) expressed the opinion that a "continental climate" was favorable to the formation of a small grain of high nitrogen content. Schindler (19) concluded that a warm, moist climate prolonged the kernel development, resulting in a high starch and low protein wheat. Melikov (13) gave heavy rainfall as the cause for low nitrogen content in wheat. LeClerc (9) deduced that the differences in composition for any one locality from season to season was due to varying weather conditions. The differences in composition of wheat analyzed by him for different localities were pronounced. Williams (41) stated that variation in protein was independent of variety and of soil treatment. He ascribed it to seasonal influences. When the wheat was small or shrunken the protein content was high and vice versa. Thatcher, in a study correlating the protein content of wheat with the rainfall in various districts from which samples were taken, observed that the protein content varied inversely with the total rainfall (35). According to Deherain (5), slow ripening was favorable to a heavy yield of wheat with average gluten content, while hot summer conditions were favorable to rapid ripening and high gluten content.

LeClerc (9, *p.* 202), in making a comparative study of the composition of wheat grown under arid and humid conditions, concluded from his experiments that the nitrogen content is higher in the wheat grown on the arid and semiarid land than it is found in wheat grown on humid and irrigated land. In other words—

an excessive amount of rainfall or irrigation is always accompanied by a crop containing a very low percentage of protein.

#### IRRIGATION WATER

Investigations pertaining to the influence of water on composition of grain have been carried on over a comparatively long period. The irrigation investigations in charge of the Utah Agricultural Experiment Station are especially valuable, and show how the nitrogen content of the grain is increased through the judicious use of water. The report of a progressive study of the nitrogen content of grain by Widstoe (37) in 1921 shows that large applications of water may increase slightly the percentage of protein in wheat. The percentage of nitrogen in spring wheat decreases with the increased use of water, ranging from 15.26 per cent protein for 20 inches to 26.7 per cent protein for 4.63 inches. With applications of water ranging from 8.89 inches to 40 inches, the variation in nitrogen content is found to be irregular, but the 40-inch application of water shows higher protein than is found in the wheat receiving either 21 or 30 inches of water.

The time of applying the water appears to be very important, since Widstoe and Stewart (38) find that the protein content of wheat decreased from 18.05 per cent to 15.98 with the increased use of water after the middle of July. When water is applied before the first of July, the protein content of the wheat is increased. Stewart and Greaves (28) concur with LeClerc (9, *p.* 202) that wheat grown on arid non-irrigated land contains more protein than is found in wheat grown on adjoining irrigated land. Prianischnikov (17) finds that high per-

centages of water in the soil lowers the nitrogen content of the grain. He also finds that the growing period for the wheat, undoubtedly due to a weaker soil solution and especially the concentration of nitrates which are inadequate for the best development, is shortened with the use of an increased water supply. Von Seelhorst and Krzymowski (21) also find that the ripening period may be shortened by keeping the water content of the soil up to 85 per cent of its capillary capacity.

Preul (16) studied the influence of variable amounts of water on the composition of wheat grown on soil rich or poor in fertility. He found that the nitrogen content of the wheat was high when the application of water was kept constantly low. A lack of water during the late stages of growth caused a comparatively low nitrogen content in the grain.

Shutt (24, 25) states that the wheat grown on newly cleared scrub land compared with wheat grown on fallow or cultivated land is physically softer, "piebald," more starchy, and lower in nitrogen content. This finding, he believed is due to the larger amount of water contained in the newly cleared scrub land, causing a prolongation of the growing period of the plant.

#### SOIL AND FERTILIZERS

The soil as a factor in influencing the composition of wheat has been studied. Thatcher (32), LeClerc (11), and Shaw and Walters (22) are of the opinion that the composition of soil has very little, if any, influence on the composition of grain. Shaw and Walters state that the soil nitrogen content has little, if any, direct influence upon the nitrogen content of grain and that the factor of climate is sufficient to entirely overshadow the soil factor. In connection with these experiments, the work of other investigators on the influence of fertilizers should not be overlooked. Snyder (26) states that—

increasing the amount of nitrogen in the soil, increases the amount of nitrogen in the grain.

Ames (1) shows that, without exception, application of nitrogen fertilizer increased the nitrogen content of the grain. The proportion of phosphorus, potassium, and nitrogen in the wheat plant is increased by the addition of these elements to the soil. Whitson, Wells, and Vivian (36) believe that under the same seasonal conditions the most important factor in causing variation in the composition of crops is the amount of nitrates in the soil. On the other hand, Soule and Van Atter (27) state that the use of fertilizers on the growing of wheat in a rich soil did not appreciably increase the protein content.

#### INVESTIGATIONAL WORK

##### I. FACTORS AFFECTING THE NITROGEN CONTENT OF WHEAT

###### EFFECT OF WIDTH OF ROWS ON NITROGEN CONTENT OF WHEAT

In the earlier investigational work undertaken at this experiment station on the influence of length of growing season, soil, or climatic condition on the percentage of nitrogen in wheat, methods of preparing the soil and seeding were followed which were not considered a part of the investigation but which undoubtedly played a very important part in the results obtained.

It was found that the percentage of nitrogen in the fall-sown wheats was only slightly lower than that in the spring-grown wheat. These similarities in nitrogen content have indicated that there are some factors, beside the length of the growing season, which have influenced the formation of wheat of high nitrogen content. It was further found that wheat grown in the nursery plots contained more nitrogen than was found in wheat grown in variety-test plots or under practical farming conditions. The wheat grown in the nursery contained 18 per cent more nitrogen than was found in the wheat grown in the variety-test plots, and this nitrogen content was considerably higher than was found in the wheat grown under field conditions. These outstanding differences in the percentages of nitrogen pointed to the influence of the method of preparing the soil on the composition of the wheat. It was observed that the wheat grown in the nursery plots was sown in rows 18 inches apart, while the wheat grown in the variety-test plots was seeded 6 inches apart. Furthermore, the nursery plots received a very thorough cultivation, a practice which was not followed in the variety-test plots. These differences in soil treatment and seeding undoubtedly played a very important part in the development of wheat containing different amounts of nitrogen and also indicated the possible reason for the similarity of the percentages of nitrogen in the fall-sown and spring-sown wheats.

The study of the influence of cultivation on wheat sown in rows different distances apart was, therefore, undertaken. The wheat was sown in rows 6, 9, 12, 15, and 18 inches apart. That sown 6 and 9 inches apart could not be properly cultivated but represented farm methods on well-prepared soil. A sample of the same wheat grown on another plot was also analyzed for the purpose of making a comparison of the nitrogen content of the wheat grown in the nursery with that under field conditions. The results are given in Table I.

TABLE I.—Percentage of nitrogen in wheat (hybrid 143) sown in rows various distances apart and cultivated

Treatment.	Fall wheat.		Spring wheat.	
	Nitrogen.	Increase over field.	Nitrogen.	Increase over field. <sup>a</sup>
	Per cent. 2. 11	Per cent.	Per cent.	Per cent.
Field grown.....				
Nursery grown:				
6 inches apart, end rows.....	2. 30	9. 0	2. 50	18. 5
6 inches apart, middle rows.....	2. 35	11. 4	2. 56	21. 3
9 inches apart, end rows.....	2. 38	12. 8	2. 46	16. 6
9 inches apart, middle rows.....	2. 32	10. 0	2. 53	19. 9
12 inches apart, end rows.....	2. 54	20. 4	2. 58	22. 2
12 inches apart, middle rows.....	2. 48	17. 5	2. 68	27. 0
15 inches apart, end rows.....	2. 53	19. 9	2. 55	20. 8
15 inches apart, middle rows.....	2. 47	17. 1	2. 56	24. 6
18 inches apart, end rows.....	2. 66	26. 1	2. 56	21. 3
18 inches apart, middle rows.....	2. 66	26. 1	2. 77	31. 2

<sup>a</sup> Calculated using (2.11 per cent) field sample of fall wheat as a basis.

It will be noted that the smallest increase of nursery-grown over field-grown wheat is 9 per cent, while the largest increase is 31.2 per cent. The fall wheat grown in rows 18 inches apart in the nursery shows 15

per cent increase in nitrogen over that grown in rows 6 inches apart. The same comparison with spring wheat shows a 10.8 per cent increase. The amount of nitrogen in the spring wheat containing the lowest percentage of nitrogen is 6.95 per cent greater than the corresponding fall wheat. Comparing the highest percentages in spring and fall wheats, it is found that the amount in the spring wheat is greater by 4.1 per cent.

From the results in Table I it will be noted that the nitrogen content of the wheat was increased by increasing the distance between the rows. This is more noticeable in the fall than in the spring seeding. Omitting the rows of 6 and 9 inch seeding, the percentage of nitrogen in the fall and spring is only slightly in favor of the latter. Owing to the fact that the extremes of variation in nitrogen are not as marked in the spring as in the fall seeding, it is of interest to note that the fall-sown wheat shows a slightly higher nitrogen content for the wheat grown in the rows next to the end than in the middle rows, with the exception of that grown 18 inches apart, where there is no difference observed. The spring wheat shows higher results for nitrogen in the middle rows than it does for those next the end.

#### EFFECT OF VARYING AMOUNTS OF WATER AND WIDTH OF ROWS ON NITROGEN CONTENT

In a subsequent experiment, a study of the influence of varying amounts of water on the nitrogen content of wheat was made. The field was divided into two series of plots, and two varieties of wheat were grown on them. The rows were sown 6, 9, 12, 15, and 18 inches apart. The amount of water used in the experiment ranged from 8.78 inches rainfall to 12, 16, and 20 inches. The land was cultivated at various times during the growing period in order to remove weeds and maintain a mulch. The average results for nitrogen content of the wheat are recorded in Table II.

TABLE II.—Average percentage of nitrogen in wheat grown on cultivated plots receiving varying amounts of water

Variety.	Distance.	Plot I, 8.78 inches of water. <sup>a</sup>	Plot II, 12 inches of water	Plot III, 16 inches of water.	Plot IV, 20 inches of water.
	<i>Inches.</i>				
Sonora.....	6	2.37	2.36	2.23	2.22
	9	2.13	2.13	2.15	2.03
	12	2.01	2.21	2.21	2.17
	15	2.26	2.21	2.12	2.30
	18	2.53	2.46	2.31	2.48
Average.....		2.26	2.27	2.20	2.24
Hybrid 143.....	6	2.21	2.13	2.20	2.26
	9	2.03	1.88	1.88	2.12
	12	2.03	2.06	2.04	2.16
	15	2.02	2.39	1.89	2.08
	18	2.17	2.42	2.20	2.30
Average.....		2.09	2.18	2.06	2.18

<sup>a</sup> Total precipitation at Grandview in 1914 was 8.78 inches.

There are some abnormal results which undoubtedly are due to the washing of the plant food from some of the plants toward others, because the lowest results for nitrogen were obtained where the irrigation water entered the plots while the highest results were found in the adjacent rows.

In general, the composition of the wheat grown on plots receiving 20 inches of total water shows approximately as high nitrogen content as was found on any of the other plots. The spreading of the rows 18 inches apart appears to have influenced the nitrogen content of the wheat.

The following year the experiment was repeated with the exception of distributing the irrigation water, especially for the 16 and 20 inch irrigated plots, over a longer period. The purpose of this change was to prevent an excessive dilution and washing of the plant food. The results for nitrogen are recorded in Table III.

TABLE III.—Average percentage of nitrogen in wheat grown on cultivated plots receiving varying amounts of water

Variety.	Distance.	Plot I, rainfall only. <sup>a</sup>	Plot II, 12 inches of water	Plot III, 16 inches of water.	Plot IV, 20 inches of water.
	<i>Inches.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Sonora.....	6	1.96	2.20	2.41	2.22
	9	1.91	2.05	2.17	2.10
	12	1.97	2.12	2.38	2.20
	15	2.08	2.10	2.15	2.17
	18	2.44	2.21	2.21	2.29
Average.....		2.07	2.15	2.20	2.20
Hybrid 143.....	6	1.83	2.16	2.30	2.21
	9	1.81	1.81	1.93	2.10
	12	1.81	1.97	2.00	2.08
	15	2.01	2.20	1.98	2.06
	18	2.21	2.44	2.14	2.24
Average.....		1.93	2.12	2.08	2.14

<sup>a</sup> Total precipitation at Grandview in 1914 was 8.78 inches.

The wheat sown in rows 18 inches apart has evidently been benefited, since it contains more nitrogen than was found in wheat sown in rows 6 and 12 inches apart.

## II. CHANGES THAT OCCUR DURING THE DEVELOPMENT OF WHEAT

### DISTRIBUTION OF NITROGEN IN KERNEL AND PLANT

In order to know more definitely what conditions are beneficial to the production of high percentages of nitrogen in the grain it is of considerable importance to know something with regard to the amount of nitrogen in the whole plant as well as different parts of the plant, especially after the development of the kernel has commenced. For this study, plants of nearly equal lengths and weights were selected at 7-day intervals. Nitrogen determinations were made on the stems and leaves between the joints or nodes, above the top node, and on kernel and chaff. The data in Table IV show the percentages of nitrogen in the plant exclusive

of kernels, percentage of nitrogen in kernels, average weight of kernel, total and average weight of nitrogen in kernel. The data in Table V show the grams of nitrogen in the chaff, above the top nodes, below the top node, below the second node from top, and in some cases (when present) below third node, as well as total weight of nitrogen in the plants.

TABLE IV.—Percentage distribution of nitrogen in plant and kernels, Hegnauer tract

Date	Nitrogen in plant, not kernel	Nitrogen in kernel	Average weight of kernel.	Total nitrogen in kernels.	Average nitrogen in kernels.
	<i>Per cent</i>	<i>Per cent.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm</i>
July 1.....	0. 871	2. 23	0. 00402	0. 00709	0. 0000885
8.....	. 800	2. 12	. 00976	. 0155	. 000207
15.....	. 571	1. 98	. 01305	. 0155	. 000258
22.....	. 529	1. 87	. 0298	. 0484	. 000557
29.....	. 460	1. 51	. 0356	. 0334	. 000528

TABLE V.—Weight and distribution of nitrogen in wheat plant, Hegnauer tract

Date	Chaff.	Above top node.	Below top node	Below sec- ond node	Below third node.	Total plant
	<i>Gm.</i>	<i>Gm.</i>	<i>Gm</i>	<i>Gm</i>	<i>Gm</i>	<i>Gm.</i>
July 1.....	0. 0074	0. 0196	0. 00569	0. 00540	0. 00239	0. 04757
8.....	. 0076	. 0102	. 0092	. 0042	. 0029	. 0496
15.....	. 00401	. 0091	. 0056	. 0042	. 0020	. 04041
22.....	. 0048	. 0089	. 0065	. 0022	.....	. 0408
29.....	. 0022	. 0038	. 0012	. 0014	.....	. 0420

It is a well-established fact that the percentage of nitrogen in the plant proper decreases with the continuous growth of the plant. This is clearly seen from the results given in Table IV, which also shows the nitrogen percentage of the kernel decreasing with the progressive development and increased weights of the kernel when grown under field conditions.

The weight distribution of the nitrogen in the several parts of the plant furnishes us some interesting data regarding the changes that take place as the kernels develop in size. It will be seen from the data given in Table V that the nitrogen (reading down the column) moves from the lowest to uppermost part of the plant, and the nitrogen is evidently the first to be drawn upon for the formation of the kernels. At the time of the filling of the kernel the highest percentage of nitrogen is found in the part of the plant above the top node and in the chaff, and the lowest percentage of nitrogen is found in the part below the lowest node. As the nitrogen enters into the kernel, the quantity in the chaff appears to be depleted first, followed by the final draft from the straw above the top node. Therefore, if the conditions are favorable for normal development, all of the excess nitrogen in the different parts of the plant will be moved upward and transferred into the kernel.

## EFFECT OF IRRIGATION WATER ON WEIGHT OF KERNEL AND VARIATION OF NITROGEN DURING GROWTH

It is generally believed that the final filling of the kernel takes place some time immediately after the grain has been cut. This belief has been based almost entirely on theory, and there is nothing in literature of a scientific nature that informs us as to its correctness. The importance of water in assisting the transference of the nitrogen into the grain has been studied, so that we are aware of the influence of water in increasing the weight and nitrogen content of the grain in the early stages of kernel development.

The plan for the first year of the experiment was to study the nitrogen composition of the developing grain at intervals of one week. The land was divided into five different plots as follows: Plot I, receiving rainfall amounting to approximately 8.78 inches; plot II, irrigated sufficiently to make the total rain and irrigation water equal to 15 inches; plot III, given 20 inches of water; plot IV, given 25 inches of water; and plot V, given 30 inches of water. Blocks of entire plants were pulled at each period and stored. Afterwards the grain was threshed out and analyzed. The results for average weight of kernels are recorded in Table VI, and average results for percentage of nitrogen are recorded in Table VII.

TABLE VI.—Weight of grain grown with various amounts of water

Date.	Plot I, rainfall only.	Plot II, 15 inches of water.	Plot III, 20 inches of water.	Plot IV, 25 inches of water.	Plot V, 30 inches of water.
	Gm.	Gm.	Gm.	Gm.	Gm.
July 1.....	0.0069	0.0052	0.0061	0.0051	0.0064
8.....	.0151	.0148	.0144	.0136	.0117
15.....	.0262	.0263	.0262	.0238	.0230
22.....	.0322	.0335	.0375	.0347	.0346
29.....	.0333	.0356	.0407	.0379	.0398

TABLE VII.—Nitrogen in grain grown with various amounts of water

Date.	Plot I, rainfall only.	Plot II, 15 inches of water.	Plot III, 20 inches of water.	Plot IV, 25 inches of water.	Plot V, 30 inches of water.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
July 1.....	3.00	3.21	3.04	2.95	2.63
8.....	2.53	2.38	2.21	1.95	2.60
15.....	2.42	2.29	1.82	1.92	1.97
22.....	2.56	2.31	2.07	1.97	2.04
29.....	2.73	2.20	2.14	1.92	2.02

The highest average weight of the kernels for the first two weeks of development was obtained in the nonirrigated plot I. At the end of the third week the weight of the kernels were equally as high in plots II and III as in plot I. For the fourth week period and at maturity, the weight of the kernels in plots II, III, IV, and V exceeded those of plot I.

With reference to nitrogen content for the first week, plots I, II, and IV have about the same values. Wheat from plot II tested the highest and wheat from plot V analyzed the lowest for nitrogen content. In the

second week the results for nitrogen were in favor of wheat grown on plot II with plot I wheat showing nearly as much nitrogen. At the third, fourth, and maturity periods the highest nitrogen content was found in plot I wheat. Wheat from plots II, III, and IV did not change appreciably after the drop in nitrogen from the first to the second week. With the exception of the wheat grown on nonirrigated land, it should be noted that the varying amounts of water applied had not appreciably affected the nitrogen content of the mature grain.

A subsequent trial was made with two varieties of wheat for the purpose of studying the influence of varying amounts of water when distributed over a longer period of growth than in the previous experiment. The average weights of kernels in the Sonora and Hybrid 143 wheats are recorded in Table VIII.

TABLE VIII.—Weight of kernels as influenced by applying varying amounts of water over a wide period and then cultivating after water application

Variety.	Distance.	Plot I, rainfall only.	Plot II, 12 inches of water.	Plot III, 16 inches of water.	Plot IV, 20 inches of water.
	<i>Inches.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
Sonora.....	6	0.0506	0.0503	0.0503	0.0506
	9	.0499	.0516	.0507	.0520
	12	.0507	.0515	.0507	.0517
	15	.0505	.0514	.0520	.0505
	18	.0515	.0524	.0523	.0503
Hybrid 143.....	6	.0332	.0331	.0344	.0338
	9	.0329	.0326	.0335	.0327
	12	.0332	.0334	.0336	.0344
	15	.0327	.0330	.0349	.0346
	18	.0343	.0346	.0350	.0350

Apparently, the variation in amount of water used or the distance of the rows has not had any effect on the Sonora wheat, since the weights of the kernels are fairly uniform, regardless of conditions of the experiments. Hybrid No. 142 wheat increased slightly in weight in the 15 and 18 inch rows, where 20 inches of water were applied.

#### EFFECT OF WATER ON TRANSLOCATION OF NITROGEN

For the purpose of learning whether or not the placing of the wheat plant in water would affect the nitrogen content of the wheat, uniform wheat plants were selected and brought to the laboratory. One-half of the wheat heads and the grain were removed from three-fourths of the samples, and each plant and the grain removed were given corresponding numbers. The heads in the other one-fourth of the samples were not touched. The plants were divided into two lots, one-half of which were placed upright in beakers of water and the other one-half were left standing near but were not placed in water. Following the termination of the experiment, determinations of the weight and the nitrogen content of the grain in both samples were made. The results are recorded in Table IX.



TABLE IX.—*Influence of water in moving nitrogenous and nonnitrogenous material from the stems into the wheat kernel*

	Average weight per kernel.	Average percentage of nitrogen.	Average weight of nitrogen per kernel.
Out of water:	Gm.		Gm.
Whole heads . . . . .	0.0071	2.62	0.000186
Half heads . . . . .	.0077	2.38	.000183
In water:			
Whole heads . . . . .	.012	3.24	.000389
Half heads . . . . .	.015	3.23	.000487

The average weight of the kernels at the beginning of the experiment was between 0.0071 and 0.0077 gm. and the nitrogen content varied from 2.38 to 2.62 per cent. At the close of the experiment, whereas the percentages of nitrogen were higher in the whole heads than in the kernels of the half heads, the total weight of nitrogen in both instances was practically the same. The weights of the kernels and percentage of nitrogen in the kernels of the plants which were placed in water had increased. The increase in the weight of kernels and the weight of nitrogen in the one-half of the kernels left on the head was found to be proportionally greater than was obtained for the average of all the kernels left intact or, as it is termed in Table IX, the whole heads. In other words, the increased amount of material in kernels left on one-half the head if distributed to double the number of kernels would have given results similar to those found in all the kernels left intact on the head. Translocation of plant food material has been brought about, and, because of its more highly nitrogeneous character, it is believed that larger quantities of water are required to move the nitrogenous matter than are required to move the nonnitrogenous matter into the kernels. Accordingly, water should prove beneficial to high rather than low nitrogen content. The protein of wheat depends first upon the supply of available nitrogen (4, v. 10; 5) for the plant, and second upon movement of the nitrogenous material into the grain.

#### RELATION OF PERCENTAGE OF MOISTURE IN THE KERNELS TO FILLING

In addition to investigational work mentioned above, the weight of the grain, moisture, starch content, etc., were determined at the various stages of the development of the kernels. The samples were collected every other day, and observations for maximum and minimum temperature and relative humidity were recorded. The results for weight and moisture content of the kernels have been plotted in figure 1.

Apparently no filling occurs in grain having a moisture content of 40 per cent or less. The vertical parts of the curves represent measurements made during periods of desiccation.

The figures for maximum and minimum temperatures and relative humidity have not been submitted because it was impossible to show conclusive relationship between these figures and those for changes of weight of kernels during the period of development. There were periods in the development, however, where the cooler nights were favorable for greater increases in weight than was found to be the case where the warmer nights prevailed. Considering the changes in weight

of the kernel, it should be noted that there were times when very little increase in weight was obtained while at other periods considerable gain was observed. On the whole, it should be noted that the kernels of wheat kept on increasing in weight up to the period when the percentage of moisture dropped to approximately 40. This period is sometimes spoken of as the period of desiccation. The slight fluctuations on the downward curve are probably due to variations occurring in method of sampling

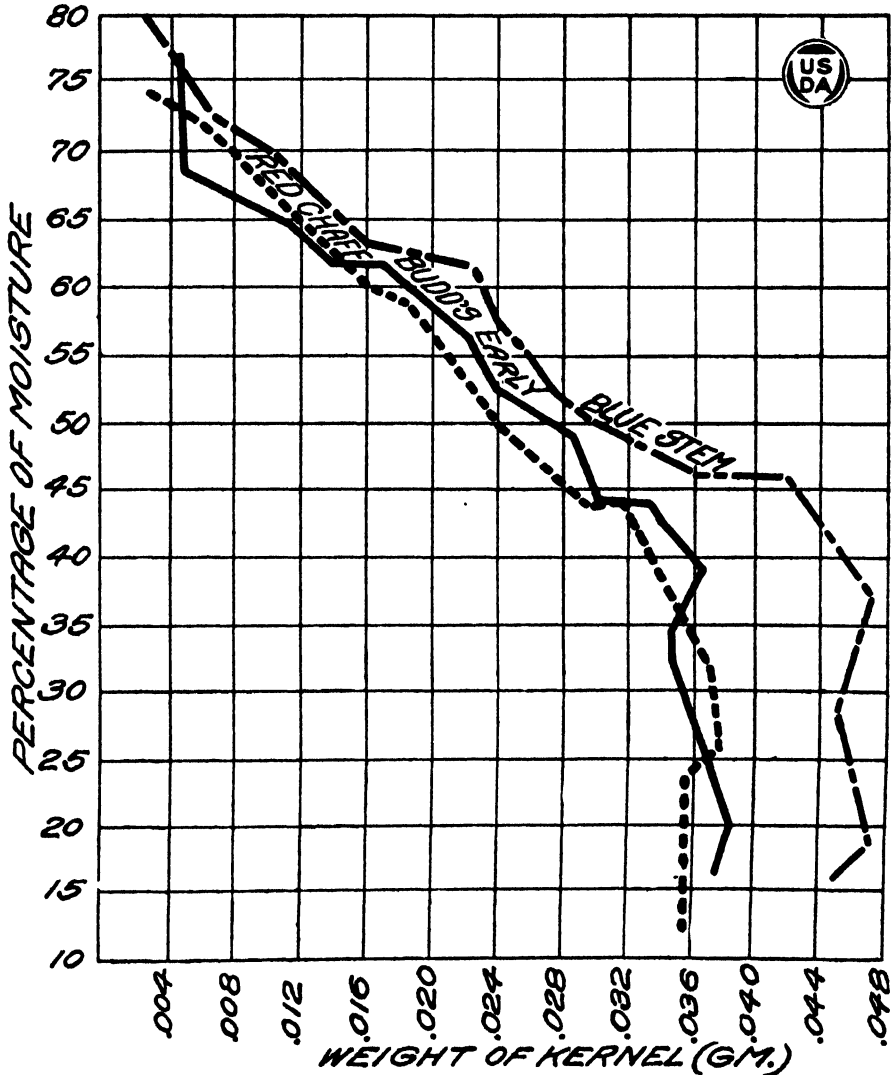


FIG. 1.—Relative moisture content of wheat kernels during growth

which at this period would be more noticeable, because the kernels were heavier, even though the errors were probably not any greater than those during the preceding periods of sampling. Any increase in nitrogen content must precede a period when the kernels contain 40 per cent or more moisture. In the period of embryo development there is considerable moisture in the grain. We also know that in the period of 60 per cent or more of moisture, the simpler forms of nitrogen material are

moving into the kernels at a very rapid rate (15) and during the period of decline in moisture content of the grain synthesize into the material known as gluten.

It is contended by Brenchley and Hall (2) that in the filling of the endosperm part of the kernel the material is uniform and always possesses the same ratio of nitrogenous to nonnitrogenous material and ash. Thatcher (33, 34) found that the ratio of protein to carbohydrates decreased with the progress of the development of the kernel. Regardless of this difference in the findings, we should not ignore the possibility that changes in composition might be brought about by abnormal conditions.

#### RELATION OF NITROGEN TO PHOSPHORUS

In the investigations by Brenchley and Hall (2) it has been shown that the ash and the phosphoric acid enter the grain simultaneously

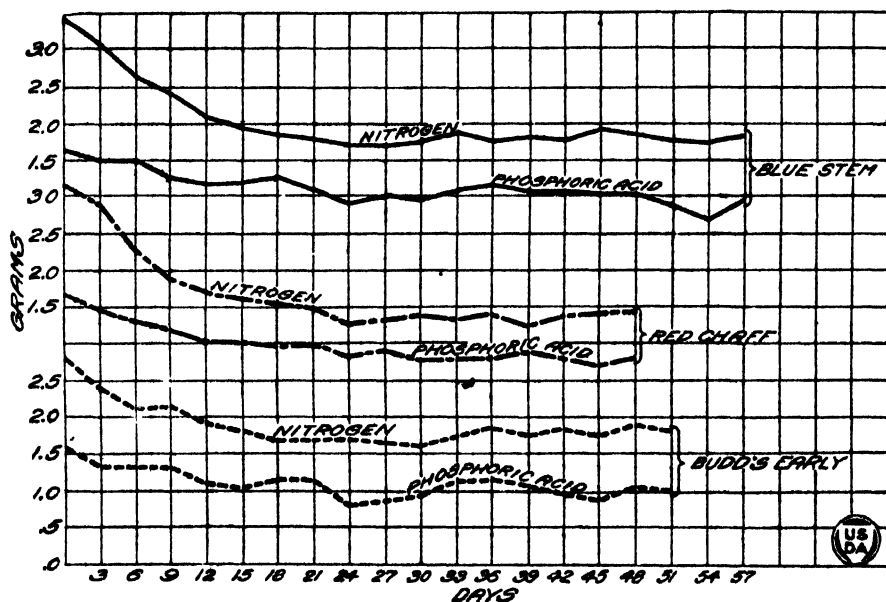


FIG. 2.—Relative time of entrance of phosphoric acid and nitrogen into three varieties of wheat

with the nitrogen. These results have been confirmed, and the graphs for nitrogen and phosphoric acid are shown in figure 2.

It is evident that not only nitrogen but phosphoric acid as well must be available in sufficient quantities in the soil if high-nitrogen wheat is to be obtained. It should be further noted that the amount of nitrogen entering into the kernels at the earliest stages of development is proportionally larger than is the case in subsequent stages of development. The embryo is developed and perfected in this early stage and undoubtedly the larger amount of nitrogen required at this period is for the construction of the embryo. Following the development of the embryo we observe that the phosphoric acid runs parallel with the nitrogen content of the kernel.

# SUMMARY

(1) Increasing the distance between the rows seemed to increase the nitrogen content of wheat grown in the nursery under nonirrigated conditions at Pullman.

(2) Hybrid 143 showed a higher percentage of nitrogen when spring-sown than when fall-sown.

(3) Increasing the distance between the rows did not seem to affect the nitrogen content of wheat under irrigated conditions at Grandview.

(4) The percentage of nitrogen in the kernel decreased as the grain matured.

(5) The nitrogen in the plant moved toward the kernel as the grain approached maturity.

(6) Irrigation did not affect the nitrogen content of wheat.

(7) Placing the wheat plant in water moved nitrogen into the kernel.

(8) Wheat kernels showed little increase in weight after the moisture declined to 40 per cent.

(9) Phosphorus entered the grain simultaneously with nitrogen except in the early stages of development.

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# RELATIVE SUSCEPTIBILITY OF CITRUS FRUITS AND HYBRIDS TO CLADOSPORIUM CITRI MASSEE<sup>1</sup>

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## INTRODUCTION

During the course of citrus-canker investigations by the authors in Alabama, only one other Citrus disease has appeared naturally with any frequency. This disease is citrus scab, caused by *Cladosporium citri* Massee (5).<sup>2</sup>

So far as the writers are aware, no one has ever attempted to make any extensive observations to determine the relative susceptibility of Citrus plants to scab. All reports so far published on this subject have been based on occasional field observations of commercial varieties. A summary of the scattered literature reveals the fact that, with a few exceptions, the Citrus plants mentioned as subject to scab by all writers can be arranged into three groups, according to their susceptibility, as follows:

1. Severely attacked.  
Sour orange.  
Rough lemon.  
Lemon.
2. Moderately attacked.  
Satsuma and other mandarin oranges.  
Lime (8, p. 82).  
Grapefruit.  
Trifoliate orange (2, p. 244).
3. Rarely attacked.  
Some few varieties of sweet orange (1; 7; 6, p. 115).

Observations on the amount of scab were made by the authors at times during the growing season over a period of four years on a representative lot of Citrus plants growing under approximately the same conditions in the canker isolation field near Loxley, Ala. They were made in connection with the authors' citrus-canker work, accounts of which have appeared (3, 4). In these publications, the type, age, and number of plants grown are given in some detail. Owing to the fact that only few of the plants were large enough to set and bear fruit, the degree of susceptibility to scab of the various plants is based on the amount of scab present on the leaves and angular wood.

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 959.



## RELATIVE SUSCEPTIBILITY TO SCAB

With the exception of *Poncirus trifoliata* (L.) Raf., all the wild relatives of Citrus so far grown in the isolation field are nonsusceptible to scab. With this exception, scab appears to be strictly limited to Citrus fruits and their hybrids. In this respect it differs from citrus-canker, which can attack a large number of Rutaceous plants.

In Table I an attempt has been made to list the majority of Citrus fruits and their hybrids grown in the isolation field, and to indicate the average relative susceptibility of these plants to scab, as has been observed at times over a period of four years under various conditions. The relative susceptibility of the various species and varieties to scab as given in the table is tentative. Many plants are quite susceptible to scab in the early spring when the first growth pushes out, while no scab appears on the foliage of the second or succeeding growths. Some plants, on the other hand, present a more or less scabby appearance during the whole season. Whether the plants placed under the first heading are nonsusceptible in a strict sense, only direct inoculations will show.

TABLE I.—Relative susceptibility of Citrus fruits and hybrids to scab

Genus and species.	Scab not observed.	Slightly suscep- tib'e.	Suscep- tib'e	Very suscep- tible.
<i>Poncirus trifoliata</i> , trifoliolate orange..		X	X	
<i>Citrus hystrix</i> , round leaf form ..		X		
<i>C. hystrix</i> , pointed leaf form ..				X
<i>Citrus</i> sp., Ichang lemon..		X		
<i>C. grandis</i> , grapefruit seedlings..		X		
Duncan ..		X		
Sullivan..		X		
Shaddock, Fla. ....				X
Pummelo, Hirado Buntan. ....	X			
Chinese, Orangedale.....				X
Marks.....				X
Indian, Roeding ..			X	
Siamese.....		X		
<i>C. sinensis</i> , orange, navel.....	X			
Temple.....	X			
Japanese No. 1 ..	X			
Japanese No. 2 ..	X			
Sekkan.....	X			
Pankan.....	X			
Tankan.....	X			
<i>C. nobilis</i> , orange, King ..		X		
<i>C. nobilis</i> var. <i>deliciosa</i> , tangerine, Cleopatra.....		X		
<i>C. nobilis</i> var. <i>unshiu</i> , Satsuma ..			X	
<i>C. mitis</i> , Calamondin.....				X
<i>Citrus</i> sp. Kanza.....				X
<i>Citrus</i> sp. Natsu-mikan ..	X			
<i>Citrus</i> sp. orange, Naranja.....				X
<i>Citrus</i> sp. orange, Narute.....				X
Citrange ( <i>P. trifoliata</i> X <i>C. sinensis</i> ).....		X	X	
Citrandarin ( <i>P. trifoliata</i> X <i>C. nobilis</i> ).....		X	X	
Citrunshu ( <i>P. trifoliata</i> X <i>C. nobilis</i> var. <i>unshiu</i> )..				X
Citrangquat (citrange X <i>Fortunella margarita</i> ).....				X
Citranguma (citrange X <i>C. nobilis</i> var. <i>unshiu</i> ).....			X	
Citrangarin (citrange X <i>C. nobilis</i> var. <i>deliciosa</i> ).....		X	X	
Citrangedin (citrange X <i>C. mitis</i> ).....		X	X	
Limequat ( <i>C. aurantiifolia</i> X <i>F. margarita</i> ).....	X			
Bigaraldin ( <i>C. aurantiifolia</i> X <i>C. mitis</i> ).....	X			
Orangelo ( <i>C. sinensis</i> X <i>C. grandis</i> ).....	X			
Oranguma ( <i>C. sinensis</i> X <i>C. nobilis</i> var. <i>unshiu</i> ).....	X			
Orangequat ( <i>C. sinensis</i> X <i>F. margarita</i> ).....		X	X	
Satsumelo ( <i>C. nobilis</i> var. <i>unshiu</i> X <i>C. grandis</i> ).....		X	X	
Clemelo ( <i>C. nobilis</i> var. <i>deliciosa</i> X <i>C. grandis</i> ).....		X		
Siamelo ( <i>C. nobilis</i> X <i>C. grandis</i> ).....	X			
Tangelo ( <i>C. nobilis</i> var. <i>deliciosa</i> X <i>C. grandis</i> ).....		X		
Siamor ( <i>C. nobilis</i> X <i>C. sinensis</i> ).....	X			
Sopomaldin ( <i>C. grandis</i> X <i>C. mitis</i> ).....	X			
Calashu ( <i>C. mitis</i> X <i>C. nobilis</i> var. <i>unshiu</i> ).....		X		
Citraldin ( <i>P. trifoliata</i> X <i>C. mitis</i> ).....	X			
Calarin ( <i>C. mitis</i> X <i>C. nobilis</i> var. <i>deliciosa</i> ).....	X			
False hybrids.....		X	X	

*Poncirus trifoliata* varies from slightly susceptible to susceptible, depending on the season. As a rule, very little scab occurs after primary infection takes place on the unfolding buds and the young shoots in the spring.

The round leaf form of *Citrus hystrix* DC. which is susceptible to canker is only occasionally attacked by scab, while the pointed leaf form which is semi-resistant to canker is very susceptible to scab. Primary infection is usually severe on young leaves and shoots, although the plants present a scabby appearance throughout the growing season.

The fact that *Citrus hystrix*, native of the Philippine Islands, where no scab has ever been found, is susceptible under our Gulf Coast conditions, leads one to assume that *Cladosporium citri* is unable to persist under Philippine conditions, which is one reason why scab has never been reported from the islands, rather than the fact that scab has never been introduced.

The Ichang lemon, possibly a natural hybrid between a lemon and pummelo, appears to be more susceptible to scab than the sour and commercial varieties of lemons.

The behavior of the plants belonging to the *Citrus grandis* group in their susceptibility to scab is quite variable, ranging from nonsusceptible to very susceptible. Whether there is a difference in the varietal resistance is not known. The fact that plants of all ages, conditions, and types were employed makes it doubly hard to draw any conclusions. Judging from observations of other investigators, the grapefruit has only within recent years been reported susceptible to scab. On the whole, it can be said that the ordinary Florida varieties, as represented by Duncan and Sullivan, are only slightly susceptible; the Florida Shaddock, very susceptible; the Indian and Chinese pummelos, susceptible; and the Siamese pummelos, slightly susceptible. The Hirado Buntan pummelo has never developed scab in the field. While there is a decided difference in scab susceptibility of the various plants in the *C. grandis* group, we do not know whether the differences are varietal or due to their individual reaction to environmental conditions which in turn influences scab susceptibility.

No scab has ever been noted on any of the plants belonging to the *Citrus sinensis* group. These observations are in common with those made by most investigators. Stevens (7) reports the Lue Gim Gong as attacked by scab in Florida, and it is the only variety of sweet orange so recorded in America. Earle (1) in Porto Rico finds that some of the round oranges are occasionally attacked by scab. Reinking (6) also reports a plant of the *C. sinensis* group subject to scab in China. On the whole, it can be stated that the sweet orange group, with an occasional exception, is nonsusceptible to scab.

Of the *C. nobilis* group, both the King orange and Satsuma are slightly susceptible to susceptible, depending on weather conditions. Most of the scab occurs during the early spring on the young leaves and small fruits. During late seasons, such as prevailed in 1915 and 1920, Satsumas are badly attacked by scab, resulting in considerable damage to the fruit. The Cleopatra tangerine, which is semiresistant to canker, evidently does not scab. It is one of the few plants promising as canker resistant which is not attacked by scab.

*Citrus mitis* Blanco is another example of a plant native to the Philippine Islands which is very susceptible to scab under our Gulf Coast conditions. Scab is usually quite severe in the early spring on the

unfolding leaves and twigs. These plants present a more or less scabby appearance throughout the season.

Yuzu or Kansu orange is a plant native of North China; in fact, it grows farther north than any other Citrus. Like *C. mitis*, it is quite resistant to canker, but very susceptible to scab. It is rather peculiar that there are here two plants, one native to the tropics, the other to North China, both somewhat resistant to canker, but susceptible to scab under Gulf Coast conditions. Scab is found on the Kansu plants throughout the growing season.

The *Natsu-Mikan*, possibly a natural hybrid similar to the tangeloes, has so far remained free from scab. The Naranja and Narute oranges, introductions from the Far East, are among the most susceptible plants in the field. These plants present an extremely scabby appearance throughout the growing season.

As a rule, all trifoliate orange hybrids are attacked to some extent in the early spring, varying in intensity, and depending somewhat on the second parent. Thus, citronshu, with Satsuma as a second parent, is more susceptible to scab than citrange with the common orange as the second parent.

The citrange hybrids vary still more in their susceptibility to scab, ranging from slightly susceptible to very susceptible. The behavior of the citrangequat is extremely interesting. Kumquat, the second parent of this cross belonging to the wild relatives, is outside the range of scab susceptibility. However, when crossed with the citrange, the resulting hybrid is very susceptible to scab, and the plants are generally scabby throughout the growing season in the field. On the other hand, it is the most promising canker-resistant hybrid that has yet been found.

Limequat has so far remained free from scab. The orangelo and oranguma have also remained clean. The orangequat is a plant similar in many respects to the Satsuma. Thus, in the type of plant and its susceptibility to canker and scab, it behaves like the Satsuma. However, why should it equal the Satsuma in its susceptibility to scab when both of its parents have been so far reported free from scab? Its susceptibility to scab must be closely related to the reaction of this plant to environmental conditions favoring scab attacks.

The bigaraldin presents the opposite extreme in that no scab has been observed on this hybrid, although both its parents, the sour orange and calomondin, are very susceptible to scab.

The mandarin orange-grapefruit hybrids as represented by satsumelo, clemelo, and tangelo vary from slightly susceptible to susceptible. No scab has been observed on siamelos. The hybrids having *Citrus mitis* as one parent either have remained free from scab or are only slightly susceptible, notwithstanding the fact that *C. mitis* is very susceptible to scab.

All the false hybrids vary from slightly susceptible to susceptible, depending on the condition of the plants.

#### SUMMARY

The range of Citrus plants susceptible to scab has been extended much beyond the few well-known groups of commercial varieties, heretofore reported. No doubt some of the plants reported as nonsusceptible may later be found to be susceptible, while many species and varieties which were not tested will also be found to be susceptible to scab.

All evidences from our observations show that scab is limited to the Citrus fruits and their hybrids, with the exception of *Poncirus trifoliata*. The species and varieties within a group vary in their susceptibility to scab. A good example of this occurs in the *Citrus grandis* or grapefruit group, and is further accentuated among the hybrids. Susceptibility to scab varies not only from season to season, depending on weather conditions, but varies even within the same season. Thus a bad scab year is determined largely by weather conditions just at the time of the formation of the leaves and fruit. In most cases, scab susceptibility narrows down to the reaction of the host plant to environmental conditions, essential to scab infection and the development of the disease. This does not necessarily mean conditions favorable to the best and most normal development of the plants, as under these conditions few of the plants are susceptible to scab. It is extremely interesting to note that in this connection a number of plants which are promising as canker resistant are as a whole susceptible to scab.

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# AN IMPROVED METHOD FOR THE DETERMINATION OF NICOTINE IN TOBACCO AND TOBACCO EXTRACTS<sup>1</sup>

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## HISTORICAL REVIEW

The two methods which are now more generally used in this country for the estimation of nicotine in tobacco and tobacco extracts are those of Kissling and the silicotungstic acid method as perfected by Chapin.<sup>3</sup> They are the only ones for this determination which have been adopted as official by the Association of Official Agricultural Chemists.<sup>4</sup> It is of interest to note in this connection that while Chapin's procedure for extracts was adopted by the association, a material change appears in the association method. This change is in the amount and manner of adding the alkali preparatory to the distillation; it is opportune, even if made unwittingly, because it tends to increase the accuracy of the method when applied to tobacco. In this connection it is important that a sufficient quantity of alkali be added in the distillation to liberate combined nicotine. The association method should emphasize this point more clearly, since it is possible in following their directions in the distillation to have the solution test alkaline due to free nicotine and yet an insufficient quantity of alkali may have been added to liberate that in combination.

Chapin describes the various procedures which have been proposed for the estimation of nicotine and the defects he found in their application, so it is not necessary to mention them here. Unfortunately, however, Chapin, in his work on the silicotungstic acid method, did not include tobacco in his studies, otherwise he probably would have found that errors may often occur in the use of this method on tobacco, as will be mentioned later. The method as outlined by him was proposed for tobacco extracts and when so applied in the present work has been found to be more satisfactory than for tobacco itself.

While it is generally recognized that the Kissling method will give good results in the hands of an experienced analyst, provided no interfering substances are present in the determination, it is nevertheless, conceded that the silicotungstic acid method will probably give more reliable and concordant results in ordinary use.

After the work reported in this paper was practically completed, the writer learned for the first time, through a mutual acquaintance, that a similar method had been published by Rasmussen,<sup>5</sup> in Denmark. As no reference to it could be found in the literature at hand, a copy was obtained

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<sup>2</sup> The author desires to thank Dr. A. M. Peter, Head of the Department of Chemistry, for helpful criticisms offered during the progress of this investigation.

<sup>3</sup> CHAPIN, R. M. THE DETERMINATION OF NICOTINE IN NICOTINE SOLUTIONS AND TOBACCO EXTRACTS. *In* U. S. Dept. Agr. Bur. Anim. Indus. Bul. 133, p. 21. 1911.

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and it was found that while Rasmussen's method is somewhat similar in principle, it is entirely different in technic from the one described in this paper. As no determinations by the former method have been made here, its accuracy is not known. Certain features of it, however, are mentioned for comparison with the writer's procedure.

The extraction of nicotine from the tobacco to which alcoholic soda has been previously added is carried on in a flask containing a mixture of equal parts of ethyl ether and petroleum ether, by frequent shaking for 5 hours. The solvent is then filtered from the tobacco through a folded filter covered with a glass plate. An aliquot is taken for acid extraction. With extracts, 3 or 4 gm. of the sample are placed in a 100 cc. flask together with 5 cc. concentrated caustic soda, 5 cc. water, and 50 cc. of the above mixed ethers. The mixture is allowed to stand 4 or 5 hours with frequent shaking, then 25 cc. of the solvent is drawn off and treated as before. In both cases the nicotine is precipitated with silicotungstic acid. Doubts might be raised as to the complete extraction of the nicotine, especially from extracts handled in the manner indicated, as compared with a Soxhlet extraction of the sample as prepared for analysis here. Furthermore, errors may arise due to evaporation when the solvents are filtered and aliquots withdrawn in the manner described. Finally, no provision is made for the prevention of emulsions often obtained in the acid washing of the ether extract of some samples and without which no method of this character is practical.

#### EXPERIMENTAL WORK

The writer, having occasion to determine the nicotine content of a large number of various grades and varieties of tobacco grown in Kentucky, found that it would be very convenient to have a method which would be more rapid than either of the official methods and equally accurate. As several determinations were to be made at the same time and facilities for making simultaneous steam distillations were not at hand, it was desirable to have a method that would, if possible, eliminate such distillations. A steam distillation of nicotine is objectionable inasmuch as it is often a long, tedious procedure that requires careful attention in order that the boiling liquid shall be maintained at a low volume to remove all nicotine.

The writer's previous experience with the Kissling method has shown that there are two principal errors to be avoided: First, the presence of ammonia in the nicotine distillate, due to its incomplete separation from the ether extract of the sample, and, second, the possible loss of nicotine in the evaporation of this ether extract previous to its distillation. The first error will, of course, give high results, due to the ammonia being titrated and calculated as nicotine, whereas the other causes low results; consequently, satisfactory figures obtained by the method may be sometimes the result of a balancing of errors. On the other hand, experience with the silicotungstic acid method in this work has shown that while it is decidedly preferable to the Kissling, nevertheless certain points in its use have to be carefully supervised in order to obtain satisfactory results. The writer's experiments show that this method as outlined by Chapin gives more satisfactory results on nicotine extracts than on tobacco. The reasons for this will be mentioned later.

Experience here has shown that the distillation as prescribed in the silicotungstic acid method, even though carried on under proper condi-

tions, is sometimes a long, tedious procedure; and, if not so conducted, will often give inaccurate results on tobacco. Accordingly, it was thought possible to substitute an ether extraction of the sample for the steam distillation of it in the silicotungstic acid method, in order to obtain the nicotine for precipitation. Afterwards, the nicotine could be extracted from the ether by acid and determined in the manner prescribed in this method. Accordingly, considerable work has been carried on with this in view, and, after many preliminary determinations, a method has been devised which has usually been found to be more rapid than either of the official methods and, with some samples at least, gives more accurate results than the silicotungstic acid method unless the latter is carried on under carefully controlled conditions. It combines the good features of both official methods and eliminates an undesirable one common to both, namely, the steam distillation. The method is as follows:

#### REAGENTS

Alcoholic sodium hydroxid solution: Dissolve 6 gm. of sodium hydroxid in 40 cc. of water and 60 cc. of 90 per cent alcohol.

Silicotungstic acid solution: Prepare a 12 per cent solution of the silicotungstic acid having the formula  $4\text{H}_2\text{O}$ ,  $\text{SiO}_2$ ,  $12\text{WO}_3$ ,  $22\text{H}_2\text{O}$ . (There are several silicotungstic acids. The acids  $4\text{H}_2\text{O}$ ,  $\text{SiO}_2$ ,  $10\text{WO}_3$ ,  $3\text{H}_2\text{O}$  and  $4\text{H}_2\text{O}$ ,  $\text{SiO}_2$ ,  $12\text{WO}_3$ ,  $20\text{H}_2\text{O}$ , do not give crystalline precipitates with nicotine and should not be used.)

Ethyl ether of quality equal to U. S. P. concentrated.

Dilute hydrochloric acids (1 to 4) and (1 to 1,000).

#### DETERMINATION

Weigh 1 gm. of tobacco extract (high grade 40 per cent) or more of extracts containing less nicotine; or 5 gm. of finely powdered tobacco which has been previously dried at room temperature or slightly above, to permit powdering, and put into a beaker or, preferably, a porcelain dish. Add 2.5 cc. of the alcoholic sodium hydroxid to the tobacco powder or about 5 cc. to the extract; it is necessary that enough alcoholic sodium hydroxid be added to liberate the nicotine. Follow in the case of extracts with a sufficient quantity of pure powdered calcium carbonate to form a moist but not lumpy mass. Mix thoroughly with a pestle or spatula, transfer to a suitable container in a Soxhlet extractor and exhaust for about 5 hours with ether. (Probably another solvent could be substituted for this purpose.) It is important that the temperature of the cooling water during the extraction should not be much over  $20^\circ\text{C}$ ., and sufficient solvent should be used in order that its volume in the extraction flask should never be less than about 25 cc. After the extraction is made, the exhausted sample is removed and the excess of ether recovered, but the volume of ether extract should not be permitted to go below the above minimum or more if necessary to keep the extractive matter in solution at ordinary temperature. (If the temperature of the cooling water is too high and the volume of solvent in the flask becomes too low during the extraction, there may be a small loss of nicotine from samples of high percentage.)

The ether extract is transferred to a separatory funnel, preferably with a short stem, the extraction flask washed with a little ether and finally about twice with hydrochloric acid (1 to 4) to remove all nicotine.



The extract is shaken with four to six portions (10 cc. each) of cold dilute hydrochloric acid (1 to 4). If an emulsion forms during the successive extractions with the acid, this can be prevented by adding a small amount of 95 per cent alcohol, about 1 cc. being sufficient for each separate extraction. (When it is found necessary to use alcohol, it is essential that it be added after the shaking has been completed. Further shaking should be avoided as much as possible, as one or two inversions of the funnel will break the emulsion. A minimum amount of alcohol and shaking are best, since it has been found that when alcohol is added along with the acid, followed by vigorous shaking, it has some solvent action on extraneous material that precipitates with the reagent. Consequently, alcohol should not be used unless necessary, and only in the manner described.) The successive extractions are run through a funnel into a 100 cc. graduated flask. A small ball of fine glass wool may be placed in the neck of the funnel. The extraction should be continued until a few drops of the acid solution give no turbidity with the reagent. The funnel is carefully washed with water and the volume made to 100 cc. An aliquot corresponding to about 0.25 gm. or more of extract or 1 to 2 gm. of tobacco, depending on the amount of nicotine present, is placed in a beaker and diluted to 400 to 500 cc. volume with distilled water. A sufficient quantity of dilute hydrochloric acid (1 to 4) is then added so as to have at least 3 cc. or more present, to each 100 cc. of liquid, including the quantity of acid contained in the aliquot. At this point, the solution should test distinctly acid with a few drops of methyl orange; if not acid, more should be added. There is then added, with constant stirring, 1 cc. of the silicotungstic acid reagent for each 0.01 gm. of nicotine supposed to be present. Stir thoroughly for about 3 to 5 minutes or until the precipitate is crystalline and settles quickly. To insure an excess of the reagent, a few drops are added to the clear supernatant liquid and the absence of any precipitate noted. The solution is allowed to stand at least 18 or preferably about 24 hours. The precipitate is filtered on an ashless filter (C. S. & S. No. 590 or similar quality) and preferably through a Hirsch or Buchner funnel by decanting first the clear liquid and testing a few drops of it with a little nicotine solution to insure an excess of reagent. By decanting practically all the clear solution the filtration is more quickly made. (In case of very small precipitates, the addition of a small amount of ashless filter paper pulp to the precipitate at the time it is to be filtered will insure a clear filtrate.) When the precipitate is transferred to the filter, if the filtrate should be turbid, it is filtered again until clear. If, at the beginning, the precipitate is made crystalline by proper stirring, the filtration is made quickly and without trouble. The precipitate is thoroughly washed with cold dilute hydrochloric acid (1 to 1,000). Transfer the precipitate, without removing from the paper, to a weighed platinum crucible, dry carefully at low heat until the paper is charred, then ignite over a Bunsen burner until all carbon is eliminated. Finally, heat over a Teclu or Meker burner for not over 10 minutes, or over a moderate blast for about 5 minutes. It is not advisable to prolong the final heating beyond the time mentioned, because of a very small but continued loss at this temperature. The weight of the residue multiplied by 0.114 gives the weight of nicotine present in the aliquot used.

In the development of this method, the following experiments were made to test important features of the work:

ABSENCE OF FOREIGN MATERIAL, IN THE ACID SOLUTION OBTAINED FROM THE ETHER EXTRACT WHICH MIGHT PRECIPITATE WITH THE REAGENT

As experiments had shown that such material would more likely be obtained from tobacco than from extracts, aliquots of the same acid solution of tobacco were compared. One aliquot was distilled and determined by the silicotungstic acid method while the other aliquot was handled at this stage as described in the method above. The results are given in Table I.

TABLE I.—Percentage of nicotine in tobacco (moisture free)

No.	Chapin's silicotungstic acid method.	Improved method.
61422 . . . . .	1. 29	1. 33
61423 . . . . .	2. 17	2. 23
61426 . . . . .	0. 70	0. 75
61436 . . . . .	7. 26	7. 30
61437 . . . . .	5. 47	5. 51
Average . . . . .	3. 38	3. 42

It is apparent from Table I that in the acid solution obtained from the ether extract of these samples, no foreign material is present which might precipitate with the reagent and materially affect the results, unless it is volatile with steam.

THE AMOUNT OF ALCOHOL USED IN THE MANNER PRESCRIBED IN THE METHOD APPARENTLY DOES NOT HAVE ANY APPRECIABLE DETRIMENTAL EFFECT

Duplicate determinations were made on different tobaccos, in one of which alcohol was used as prescribed, while in the other it was omitted. The results are given in Table II.

TABLE II.—Percentage of nicotine in tobacco (moisture free)

No.	Alcohol used.	No alcohol used.
80236 . . . . .	6. 94	6. 80
80237 . . . . .	5. 98	6. 02
80238 . . . . .	6. 35	6. 30
80239 . . . . .	5. 72	5. 82
80273 . . . . .	2. 98	2. 87
80274 . . . . .	2. 10	2. 05
80275 . . . . .	1. 79	1. 77
80276 . . . . .	0. 97	0. 9
80382 . . . . .	7. 77	7. 7
80383 . . . . .	7. 04	6. 94
80384 . . . . .	5. 78	5. 70
Average . . . . .	4. 86	4. 82

Apparently alcohol used in the quantity and manner prescribed in the method has very little, if any, effect on the results. The use of alcohol would be more likely to affect the results in the analysis of tobacco than in that of extracts. Experience has shown that it is not usually required for extracts and is not always necessary for tobacco.

Alcohol has been found, however, to have a solvent action for extraneous matter contained in the ether extract of some tobaccos if it is added with the acid and thoroughly shaken, as is done in the acid extraction. For example, two of the samples in Table II, when alcohol was used in this manner, gave the following results: No. 80236 = 7.37 per cent and No. 80237 = 6.32 per cent nicotine. On the other hand, it has been repeatedly found that when as much as 5 cc. alcohol is added to an aliquot after the acid extraction is made, identical results are obtained as compared with another aliquot containing no alcohol. However, it should not be used except where it is necessary and then only in the manner prescribed.

When a comparison was made of this method with Chapin's silicotungstic acid method, it was found that higher, and in some samples of tobacco abnormally higher, results were obtained by the latter method. Much better agreement, however, was shown with extracts. Steam distillations showed that whereas not over 750 cc. of distillate was usually sufficient to obtain the nicotine from extracts, a much larger volume was generally required for tobacco, notwithstanding the fact that a smaller amount of nicotine might be present in the larger distillate. Again, it was found essential that the solution in the distilling flask should be maintained at about 15 to 25 cc. in order to distill over the nicotine in a minimum volume of distillate. If the volume of liquid greatly exceeds this extreme difficulty is met with in distilling the nicotine, especially from tobacco. For example, tests with negative results have been made for nicotine in a distillate coming from a comparatively large solution, although further concentration to the optimum volume showed its presence. Another interesting fact observed in the steam distillation was that the nicotine results obtained by its use, especially with tobacco, were largely influenced by the amount of alkali used in the distillation. For instance, it was found that where 2 gm. of sodium hydroxid was used in the distillation higher results were always obtained than when smaller amounts were employed, despite the fact that in the former case the nicotine usually came over in a smaller volume of distillate. In all distillations the liquid in the distilling flask was alkaline, and sufficient alkali was present to liberate combined nicotine. It has been shown in this connection that the larger amount of alkali liberates some volatile compound other than nicotine which comes over in the distillate and is precipitated by the reagent. This substance occurs in variable quantity in tobacco, and a distillate containing it generally has some odor and color. The compound does not give the characteristic test for nicotine, but forms with silicotungstic acid a dirty white precipitate which becomes pinkish on standing. Where this substance occurs in appreciable quantity it can be obtained by distilling with an excess of alkali (2 gm.) the exhausted tobacco obtained from the ether extraction, or, after a weak alkaline distillation is completed, a further addition of alkali up to the above amount and continued distillation will show its presence. There is no doubt but that the excess of alkali and heat used in the distillation acts to a greater or less extent on the tobacco residue

contained in the small volume necessary to liberate the nicotine, and forms a volatile product or products which are found in the distillate, precipitate with the reagent and affect the results. Further work will be done to determine, if possible, the nature of this reaction.

The following precautions are therefore necessary in the distillation: (1) Use the minimum amount of alkali necessary to liberate all the nicotine; (2) keep the volume of liquid in the flask at about 15 to 25 cc. to facilitate the distillation of the nicotine; (3) continue the distillation until a few cubic centimeters of the distillate shows no opalescence when treated with a drop of dilute hydrochloric acid and a drop of the silicotungstic acid. Under such conditions, however, a large volume of distillate will sometimes be obtained, as shown in Table III.

TABLE III.—Percentage of nicotine in tobacco (moisture free); effect of different amounts of NaOH in distillation of 5 gm. sample on the volume of distillate necessary to liberate the nicotine.

No.	2 gm. NaOH.	Volume of distillate.	Final test for nicotine.	Less than 2 gm. NaOH.	Volume of distillate.	Final test for nicotine.
61469	7.55	900	Absent. . . . .	<sup>1</sup> 7.38	1,500	Absent.
				<sup>1</sup> 7.29	1,500	Do.
				<sup>2</sup> 6.90	2,900	Do.
61537	6.33	900	do . . . . .	<sup>2</sup> 5.67	1,800	Present.
				5.98	2,400	Absent.
80073	7.85	1,800	do . . . . .	<sup>1</sup> 6.76	1,000	Do.
	7.83	1,800	do . . . . .	<sup>1</sup> 6.65	1,000	Do.
80144	. . . . .	. . . . .	. . . . .	<sup>2</sup> 7.32	2,100	Present.
				<sup>2</sup> 7.51	2,700	Absent.

<sup>1</sup> NaOH used in distillation, 0.25 gm.

<sup>2</sup> NaOH used in distillation, 0.15 gm.

#### DIRECT PRECIPITATION OF NICOTINE

It was thought that it might be possible with some extracts, provided no interfering substances were present, to make a direct precipitation of the nicotine and thereby eliminate the distillation and ether extraction necessary in the other methods. Experiments were made, therefore, by taking a weighed sample of extract, diluting it to a definite volume and directly precipitating the nicotine in an aliquot with the reagent in the manner prescribed in the improved method as to volume, acidity, and handling of the precipitate. While the precipitates were often colored, very satisfactory results were obtained on most of the extracts, although precipitable matter in others proved to be detrimental. The results obtained are given in Table V.

This method was also tried on tobacco by making an aqueous or weak alkaline extraction, filtering and using an aliquot as described in the preceding paragraph. It was not found applicable, however, as shown by the determinations below, on two samples from Table III. No. 61469 gave 9.13 per cent nicotine by an alkaline extraction and No. 61537 gave 7.53 per cent by an alkaline and 7.37 per cent nicotine by an aqueous extraction.

The results obtained by the improved method on tobacco and extracts in comparison with Chapin's silicotungstic acid method, together with those found by the latter when variable amounts of alkali were used in the distillation, are given in Tables IV and V.

TABLE IV.—Percentage of nicotine in tobacco (moisture free).

No.	Chapin's silicotungstic acid method.		Improved method.
	2 gm. NaOH used in steam distillation of 5 gm. sample.	Less than 2 gm NaOH used in steam distillation of 5 gm. sample.	
61469	7.55	<sup>1</sup> 7.38	6.99
		<sup>1</sup> 7.29	7.01
		<sup>2</sup> 6.90	7.00
61537	6.33	<sup>1</sup> 7.19	5.98 6.18
		<sup>2</sup> 5.98	
80073	7.85 7.83	<sup>1</sup> 6.76	6.08
			6.70
	7.84	<sup>1</sup> 6.65	6.88
80074	6.37	6.71	6.79
80076	5.32	<sup>1</sup> 6.19	6.01
80117	3.67	<sup>1</sup> 5.09	4.92
80121	3.97	.....	3.37
80126	2.71	.....	3.68
80129	4.57	.....	2.48
80130	4.50	.....	4.43
80134	1.75	.....	4.24
80144	.....	<sup>2</sup> 7.51	1.55
80195	.....	<sup>2</sup> 7.77	7.67
80263	.....	<sup>1</sup> 2.56	7.83
			2.57
			2.58
			2.58

<sup>1</sup> NaOH used in distillation, 0.25 gm.

<sup>2</sup> NaOH used in distillation, 0.15 gm.

TABLE V.—Percentage of nicotine in tobacco extracts and products

No.	Brand.	Guaranty.	Direct precipitation method.	Chapin's silico-tungstic acid method. <sup>1</sup>	Improved method.
43889 <sup>2</sup>	.....	16.42	18.77	<sup>3</sup> 17.31 17.44	15.96 16.71
80054	"Nico-Fume".....	40.00	41.60	<sup>3</sup> 17.38 40.78	16.34 40.77
80055	"Hammond's Tobacco Extract".....	4.00	4.26	4.27	4.25
80056	"Nikoteen".....	30.00	31.67	30.85	30.78
80064	"Thompson's Rose-Nicotine".....	5.00	5.13	<sup>5</sup> 5.18 <sup>5</sup> 5.26	<sup>4</sup> 4.89 <sup>4</sup> 4.89
80065	.....	.18	.72	5.22 .13	4.89 .13
80066	.....	3.57	4.78	<sup>5</sup> 3.70 <sup>5</sup> 3.82	<sup>4</sup> 3.51 <sup>4</sup> 3.51
80356	"Hall's Nicotine Solution".....	40.00	40.43	<sup>3</sup> 3.76 <sup>3</sup> 40.62	3.51 39.69
80357	"Hall's Nicotine Sulfate".....	40.00	40.15	<sup>3</sup> 39.82	39.88
40358	"Hall's Nicotine Fumigator".....	12.50	.....	<sup>3</sup> 13.26	13.18
80823	"Black Leaf 40".....	40.00	40.54	40.00	40.13
80824	.....	.....	1.64	<sup>6</sup> 2.47 2.50	1.65
80826	.....	.....	94.63 94.70	<sup>7</sup> 2.49 <sup>7</sup> 93.73 <sup>7</sup> 93.75 <sup>7</sup> 94.60	93.42 93.60 94.14
80828	.....	40.00	40.61 40.73	94.67 <sup>8</sup> 94.03 <sup>8</sup> 39.74 <sup>8</sup> 40.19	93.72 40.39 40.88
80829	"Black Leaf 40".....	40.00	40.67 42.21	<sup>8</sup> 39.97 <sup>8</sup> 41.87	40.64 42.09

<sup>1</sup> In this method, the following weights of sample were distilled with 2 gm. NaOH unless otherwise indicated.

Samples containing 50 per cent or more of nicotine, 0.6 gm.

Samples containing 30 to 50 per cent of nicotine, 1 gm.

Samples containing 3 to 30 per cent of nicotine, 2 gm.

Samples containing less than 3 per cent of nicotine, 5 gm.

<sup>2</sup> This extract had been prepared for several years and was supposed to contain 16.42 per cent of nicotine, 2.78 per cent of pyridin and 2.37 per cent of ammonium chlorid. The nicotine guaranty is probably only approximate. The results obtained for nicotine by all methods include any pyridin that may have been present in the washed precipitate.

<sup>3</sup> NaOH used in distillation, 0.60 gm.

<sup>4</sup> Duplicates from same ether extraction.

<sup>5</sup> NaOH used in distillation, 0.15 gm.

<sup>6</sup> NaOH used in distillation, 0.24 gm.

<sup>7</sup> NaOH used in distillation, 0.90 gm.

<sup>8</sup> NaOH used in distillation, 0.30 gm.

## SUMMARY

(1) A method has been devised for the determination of nicotine in tobacco and tobacco extracts which has been found to be as rapid and as accurate as the silicotungstic acid method and more satisfactory on tobacco. The other standard method commonly used for nicotine is the Kissling.

(2) The new procedure eliminates an undesirable feature, namely, the steam distillation, common to both of the above methods.

(3) It has been shown that special precautions should be taken in carrying on the distillation in the silicotungstic acid method. Under proper conditions very good results were usually obtained by its use; otherwise, serious errors were often found when working on tobacco.

(4) A direct precipitation method which obviates a preliminary ether extraction or steam distillation to obtain the nicotine, has also been tried and fairly satisfactory results were obtained on most of the tobacco extracts employed. It was not found, however, to be applicable to tobacco and a few extracts.

# NUTRITIVE VALUE OF MIXTURES OF PROTEINS FROM CORN AND VARIOUS CONCENTRATES<sup>1</sup>

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## INTRODUCTION

The nutritive value of mixtures of peanut and soy-bean flours as supplements to wheat-flour proteins has been shown in previous publications (4, 5)<sup>2</sup> from this laboratory. This paper is a continuation of these studies with whole yellow corn. In addition to the peanut and soy-bean flours as protein supplements to corn are also included meal made from tomato-seed press cake and coconut meal made by both the solvent and expression processes. Data on both the chemical and nutritional studies of the proteins from tomato seed and coconut have already been published (3, 6, 7, 8, 9). Each of the concentrates mentioned has been found adequate for the normal growth of albino rats as the sole source of protein in otherwise complete diets.

It has been shown by several investigators that whole corn is not adequate for normal growth. Feeding experiments with zein, a protein which constitutes more than one-half of the total proteins in maize, have shown it to be deficient in lysine and tryptophane—amino acids which are essential for the growth of animals. Experiments also have been recorded which show that when corn proteins are fed at a high enough intake level—as, for example, in corn gluten—normal growth can be secured.

Whole corn constitutes a large part of the ration of many animals and it is of practical importance to the feeder to know whether the addition of small quantities of protein concentrates such as coconut meal, tomato-seed, press cake, soy-bean and peanut flour will result in a protein mixture which will meet the nutritional requirements for normal growth. Our experiments with these concentrates show that a mixture of 25 parts of tomato-seed press cake, soy-bean flour, or peanut flour with 75 parts of corn satisfies the protein requirement for the normal growth of white rats. In the case of the coconut meal, equal parts were necessary to secure the same result. This may not have been due, however, to inferiority in the nutritional value of the coconut-meal proteins, but rather to the bulky character of the meal caused by its higher content of crude fiber. Chemical studies of the proteins of these concentrates have shown that they are relatively high in lysine and tryptophane, and are therefore well suited to supplement the corn proteins which are deficient in these amino acids.

## EXPERIMENTS WITH WHOLE YELLOW CORN MEAL

Two diets containing 7.2 per cent of protein in which the proteins came solely from whole yellow corn were used. Diet 1 contained 16 per cent of butter fat. Diet 2 was made up of the same composition, with

<sup>1</sup> Accepted for publication Nov. 24, 1922.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 977-978.



the exception that lard was used in the place of butter fat. The growth curves in Chart 1 show that about equally poor growths resulted with both of these diets, an average gain in weight of only 0.73 gm. per gram of protein intake being obtained. Although diet 2 contained no source of vitamin A other than the yellow corn (unless the lard may have contained some), growth was obtained at approximately the same rate as when diet 1 with the butter fat was used. No indication of xerophthalmia was observed. These facts indicate the presence of vitamin A

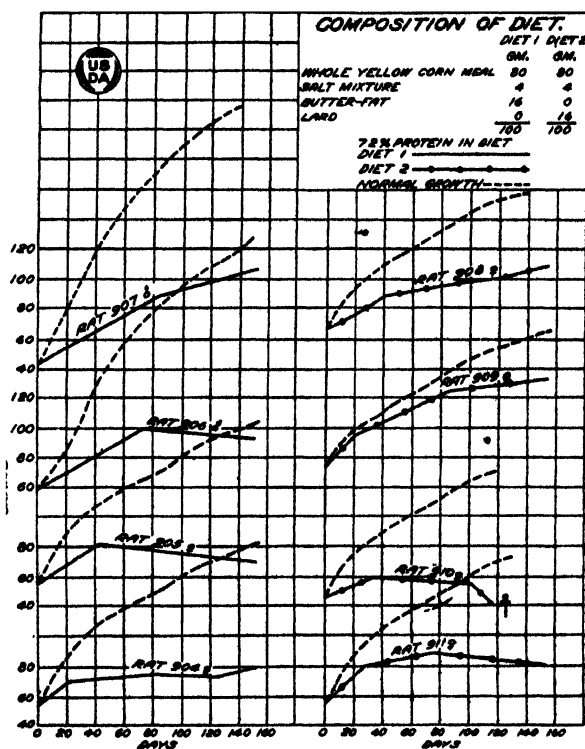


CHART 1.—Curves showing the poor rate of growth of young albino rats fed on a diet the proteins of which came solely from whole yellow corn.

in yellow corn, as has previously been shown by Steenbock and Boutwell (13).

#### EXPERIMENTS WITH CORN AND TOMATO-SEED PRESS CAKE

Studies made in this laboratory (8) have shown that tomato-seed press cake contains nearly 37 per cent of protein and that the nutritionally essential amino acids are well represented in these proteins, being relatively high in arginine, lysine, and cystine. Qualitative tests also show the presence of tryptophane. The nutritional adequacy of these proteins, as shown by chemical studies, has been confirmed by feeding experiments (3).

On a diet in which the proteins were furnished by 75 parts of whole yellow corn meal and 25 parts of tomato-seed press cake, growth at the normal rate was secured (Chart 2). On such a diet, the tomato-seed press cake furnished 7.4 per cent, and the corn 5.4 per cent of protein,

Decreasing the protein level to 7.2 per cent, but keeping the proportion of tomato-seed press cake to corn the same, resulted in a rate of growth somewhat below the normal. This was better, however, than the diet in which corn alone was used to furnish the same amount of protein. An average gain in weight of 0.94 gm. per gram of protein intake was obtained during an 11-week period on this mixture. With the corn alone, an average gain of only 0.73 gm. was secured. The low level of the protein intake was responsible for a rate of growth somewhat below normal. By using a diet somewhat below the minimum requirement of protein for normal growth, the maximum gain in weight per gram of protein intake is obtained. Such a diet affords a better basis for a comparative study of the growth-promoting value of different proteins.

#### EXPERIMENTS WITH CORN AND COCONUT PRESS CAKE

Diets in which the proteins were obtained from mixtures of equal parts of coconut meal and whole yellow corn enabled albino rats to

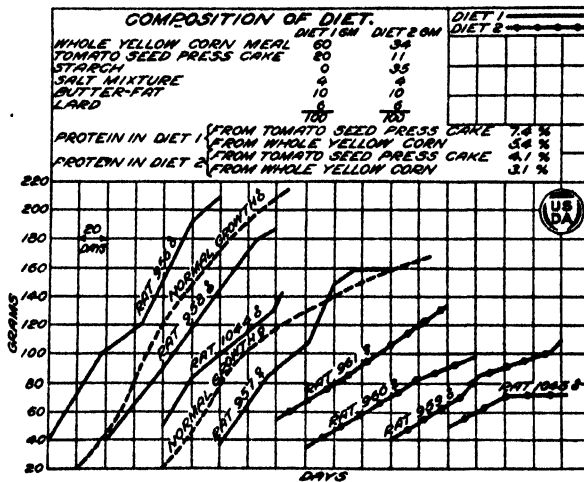


CHART 2.—The plain continuous lines represent the rate of growth of rats on diet 1, containing 5.4 per cent of protein from corn, supplemented with 7.4 per cent of protein from tomato-seed press cake; the growth represented by the continuous dotted lines was made on diet 2, of which 3.1 per cent of the proteins came from corn, and 4.1 per cent from tomato-seed press cake.

grow at the normal rate. On such a diet, the coconut meal furnished 8.4 and the corn 3.6 per cent protein, respectively. This experiment shows that the coconut proteins supplement corn proteins, since better than normal growth was obtained with 12 per cent of the mixture of proteins, while 13.1 per cent of coconut proteins alone, in an otherwise adequate diet, just barely sufficed for normal growth (7). When coconut meal furnished 4.2 per cent and corn 5.4 per cent protein in the diet, growth was somewhat subnormal. The coconut meal used in these diets was made both by the expression process (A) and by the solvent process (B). The processes seemed to be of equal value as regards the nutritive properties of the meal produced. The results of this experiment are recorded in Chart 3. The efficiency of a coconut-corn protein mixture for promoting growth, as shown by these experiments, is in agreement with the results reported by Maynard and Fronda (11), who found that "a mixture of corn meal protein and coconut-oil-meal protein was of

slightly higher quality than the former alone, but much better than the latter alone."

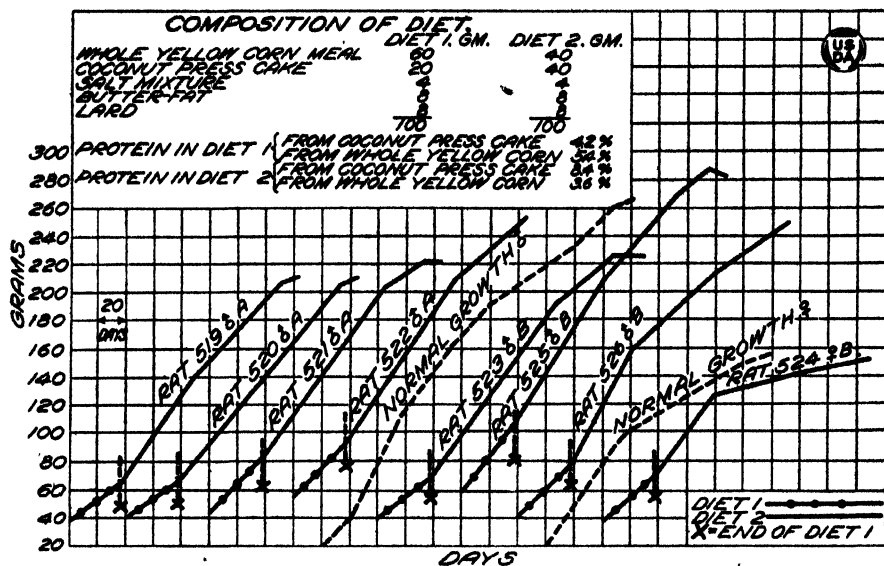


CHART 3.—Curves showing the growth-promoting efficiency of a coconut-corn protein mixture. The compositions of the diets are given on the chart.

#### EXPERIMENTS WITH CORN AND PEANUT MEAL

When peanut meal forms the sole source of protein at intake levels of from 15 to 18 per cent in diets which are otherwise complete, Daniels and

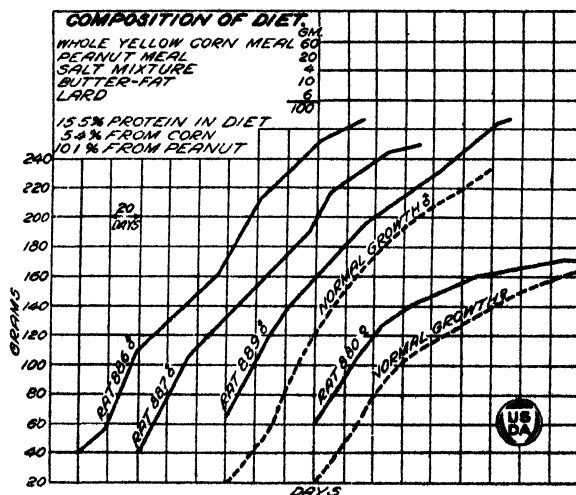


CHART 4.—These curves represent the growth-promoting value of a protein mixture, 5.4 per cent of which was furnished by corn meal, and 10.1 per cent by peanut meal.

Loughlin (1) have shown that normal growth can be secured. In our experiments normal growth was obtained with mixtures consisting of 75 parts of corn meal and 25 parts of peanut flour at a protein level of 15.5 per cent. From such a protein mixture two-thirds of the protein was

furnished by the peanut flour (Chart 4.) By keeping the proportion of the concentrate to corn the same, but lowering the protein level of the diet to 7.2 per cent, a better rate of growth was obtained than when corn

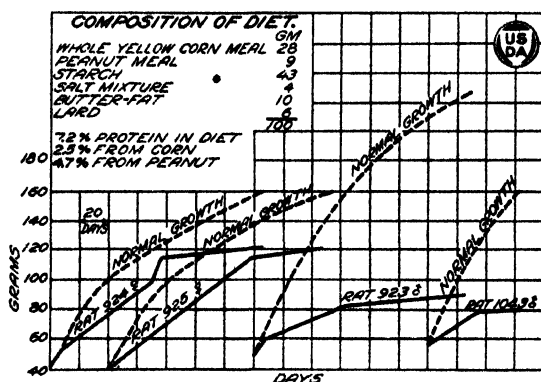


CHART 5.—Growth curves showing the supplementary value of peanut proteins when fed with corn proteins, at a low protein intake level.

alone furnished the protein in a diet otherwise adequate at the same level (Chart 5).

The average gain in weight per gram of protein in this mixture during an eleven-week period was 1.23 gm. compared with 0.73 gm. on corn alone. Rat 1043, in the above lot, was not included in the average.

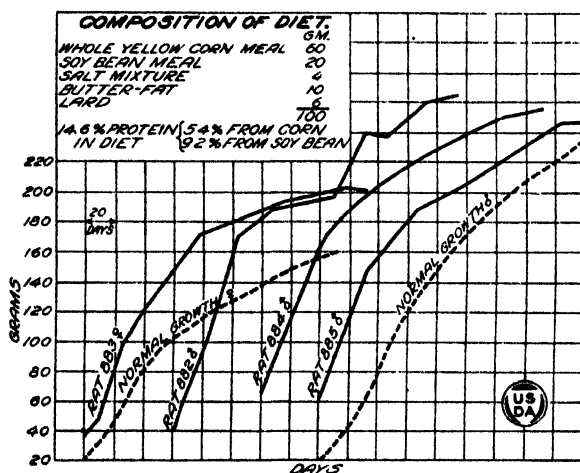


CHART 6.—These curves show the excellent growths obtained on a diet 5.4 per cent of the proteins of which was furnished by corn, and 9.2 per cent by soy-bean meal.

#### EXPERIMENTS WITH SOY BEAN AND CORN

It has been shown (12, 2) that soy-bean proteins are adequate for the normal growth of albino rats. Our experiments were made with a diet in which 25 parts of soy-bean flour replaced an equal quantity of corn meal. This diet contained 14.6 per cent of protein. Better growth than at the normal rate was obtained (Chart 6) when to such a mixture was added a suitable inorganic salt mixture, lard and butter fat. In this diet, 9.2 per cent of the protein was furnished by soy beans and 5.4 per

cent by the corn. McCollum (10) has shown that a supplementary relationship exists between corn and soy-bean proteins in the ratio of 6 per cent of the former to 3 per cent of the latter.

When the protein level of the corn-soy-bean mixture was reduced to 7.2 per cent, so that the corn furnished 2.6 per cent and soy bean 4.6 per cent of the protein of the diet, decidedly better growth resulted than when corn at the same protein level in an otherwise adequate diet was used. Rat 926 of this lot showed even a better than normal rate of growth at the end of about 95 days (Chart 7).

During an eleven-week period, the rats of this lot (excluding rat 1041, which for some reason other than a deficiency in the diet made practically

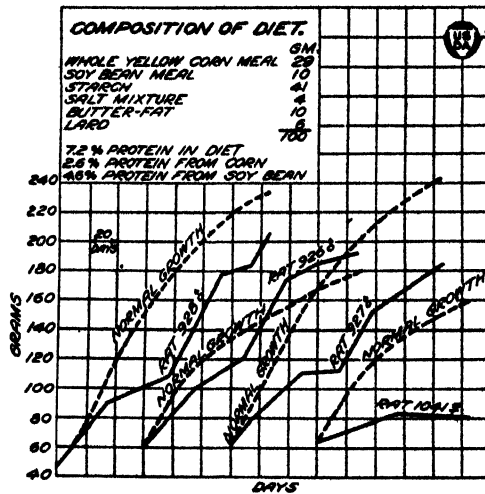


CHART 7.—Curves showing the supplementary value of soy-bean proteins when fed with corn proteins, at a low protein intake level.

no growth) made an average gain of 1.48 gm. per gram of protein consumed.

TABLE I.—Gain of body weight per gram of ingested protein

Diet. <sup>1</sup>	Protein in—			Gain per gram (11 weeks).
	Corn.	Concentrate.	Total.	
	Per cent.	Per cent.	Per cent.	Gm.
Corn.....	7.2	.....	7.2	0.73
Corn+tomato-seed press cake.....	3.1	4.1	7.2	.94
Corn+peanut meal.....	2.5	4.7	7.2	1.23
Corn+soy-bean meal.....	2.6	4.6	7.2	1.48

<sup>1</sup> These diets were made adequate with respect to the nutritionally essential factors other than proteins. (See charts.)

Although the ratio of the percentage of concentrate protein to corn protein is not exactly the same in these diets (Table I), it can safely be concluded that the growth-promoting value of the proteins of these concentrates, as supplements to the proteins of corn, is in the following order: Soy bean, peanut, and tomato seed. In the experiments with the coco-

nut press cake, no diets containing as low a protein content as 7.2 per cent were used. Therefore, a direct comparison of the nutritive value of the proteins of this concentrate with the other concentrates studied, cannot easily be made.

#### SUMMARY

Mixtures consisting of 25 parts of tomato-seed press cake, soy-bean flour, or peanut flour, and 75 parts of yellow corn meal, which contained from 12 to 15 per cent of protein, have been found to furnish proteins adequate for the normal growth of albino rats when incorporated in a diet made nutritionally adequate with respect to the dietary factors other than protein.

A mixture of equal parts of corn meal and coconut meal at a protein level of 12 per cent was found efficient for growth at the normal rate. The growth was somewhat subnormal when the proportion of protein was reduced to 9.6 per cent.

From experiments in which the mixtures of corn meal and concentrates furnished 7.2 per cent of protein, it is concluded, from the gain in weight per gram of protein consumed, that the comparative growth-promoting value of the proteins of tomato seed, peanut and soy bean, as a supplement to corn proteins, is in the order: Soy bean, peanut, and tomato seed.

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## THE MODE OF INHERITANCE OF RESISTANCE TO PUCCINIA GRAMINIS WITH RELATION TO SEED COLOR IN CROSSES BETWEEN VARIETIES OF DURUM WHEAT<sup>1</sup>

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and O. S. AAMODT, *Assistant Pathologist, Office of Cereal Investigations, Bureau  
of Plant Industry, United States Department of Agriculture*<sup>2</sup>

### INTRODUCTION

It has long been recognized that the production of economically desirable varieties of wheat resistant to *Puccinia graminis tritici* Erikss. & Henn. would be of great value. Until recently, hybridization experiments with this end in view were based upon a very incomplete knowledge of both the exact nature of the pathogene and the nature of the inheritance of resistance, and consequently, satisfactory results were not obtained. A knowledge of the mode of inheritance of certain desired economic characters of wheat together with the discovery that there are several biologic forms of stem rust of wheat has been of much importance in placing breeding studies on a definite basis. Several varieties belonging to different species or subspecies of wheat have been found to be resistant to certain biologic forms. It is hoped that by hybridization, resistance to all biologic forms may be combined in one variety. The present problem was attacked chiefly for the purpose of determining whether the resistance of two varieties, having different factors for resistance, may be combined in the progeny of a cross between them.

### REVIEW OF PREVIOUS INVESTIGATIONS

Since 1894, when Eriksson (7)<sup>3</sup> discovered biologic specialization in the black stem rust, numerous investigators (6, 9, 25) have corroborated his work. The reports of Ward, Evans, and others (32, 8, 9) indicated that biologic forms might change rather rapidly as a result of host influence but more recent work by Stakman and others (27, 30) showed that biologic forms were apparently quite stable. Previous to 1916, it was generally believed that only one form of black rust attacked wheat.

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<sup>2</sup> The authors wish to express their appreciation for the assistance rendered by Dr. H. K. Hayes and Dr. E. C. Stakman, both of the Minnesota Agricultural Experiment Station, and to thank Mr. M. N. Levine, Assistant Pathologist, Office of Cereal Investigations, U. S. Department of Agriculture, for certain biologic forms of wheat rust which he furnished.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 995-996.



However, the investigations of Stakman and others (28, 29, 21, 20, 26) have proved that there are a number of biologic forms of this rust.

A total of 37 different biologic forms of *Puccinia graminis tritici* have been isolated at the Minnesota station by Stakman and Levine (25a). No one form has been found to attack all varieties of *Triticum* species and no wheat variety has been found to be resistant to all rust forms, except Khapli emmer, a variety of *T. dicoccum*. The exact distribution of each biologic form is not known (19). Perhaps not half of the 37 forms are present in any one wheat-growing district. For example, only 14 forms had been collected up to December, 1921, in western Canada (23). However, as it is possible that some forms may spread into new districts, the problem of breeding for resistance means working for a variety resistant to all known forms of rust.

The study of disease resistance in wheat dates back over a century. According to Biffen (3), Thomas Knight, in 1815, suggested that disease-resistant varieties might be raised, and Farrar, in 1889, stated that susceptibility to rust was hereditary in wheat. Biffen (3), in 1905, crossed Red King, a variety susceptible to *Puccinia glumarum* (Schm.) Erikss. & Henn., with Burt, a resistant variety. The  $F_1$  progeny was susceptible and the  $F_2$  progeny segregated into susceptible and resistant in a 3:1 ratio. Later, Biffen (4) stated that the relatively immune hybrid forms of wheat bred true and that the immunity was independent of any discernible morphological character, that is, the factors for resistance were inherited independently of other factors. More recently, Biffen (5) reported the production of a rust-resistant wheat which likewise possessed other desirable economic characters.

Some excellent results in breeding for disease resistance in crop plants have been reported by various workers, but the problem is rarely as difficult as in the case of stem rust of wheat. Gaines (12) crossed two varieties of wheat highly resistant to bunt and obtained in the  $F_2$  some plants which were more susceptible than either parent and a large percentage which were immune. He concluded that the two varieties had different factors for resistance. Garber (13), in 1921, found that resistance to *P. graminis avenae* Erikss. & Henn. was a dominant character, a ratio of 3 resistant to 1 susceptible being obtained in  $F_2$ .

A definite grouping of the wheat subspecies according to their reaction to *P. triticea* Erikss. was made by Vavilov (31) in 1914. The use of inter-specific crosses in breeding for rust resistance is liable to some complication owing to the linkage of certain factors. In 1917, Freeman (10) reported cases of linked quantitative characters in wheat crosses. In 1919, Hayes, Parker, and Kurtzweil (16) found that in crosses of varieties of *T. vulgare* with varieties of *T. dicoccum*, resistance was dominant. In crosses of *T. vulgare* with *T. durum* susceptibility was dominant and in  $F_2$  there was strong linkage between rust resistance and the durum character. The significant fact, however, was that some crossing-over occurred. It was apparent, therefore, that there was a possibility of transferring the resistance of durum or emmers to common wheats.

Puttick (24), in 1920-21, studied the reaction of the  $F_2$  generation of a cross between a common and a durum wheat to two biologic forms of *P. graminis tritici* to which these varieties reacted reciprocally.  $F_2$  seedlings were inoculated with one rust and later the infected leaves were cut off and the plants were inoculated with a second rust form. All gradations between complete susceptibility and immunity to both

forms of rust were obtained. Some of the progeny appeared to be resistant to both forms.

More recently Melchers and Parker (22) found that a single factor was responsible for the resistance in crosses of Kanred with Marquis, Preston, and Haynes Bluestem, which were inoculated with a single form of rust. Segregation in  $F_2$  was in a 3:1 ratio of resistant and susceptible plants. The results were verified by carrying the study through the third generation.

Aamodt (1, 2), in 1921, studied the inheritance of resistance to several biologic forms of *P. graminis tritici* in a cross between Kanred, a winter wheat, and Marquis, a spring-sown variety. Kanred is resistant to a number of biologic forms whereas Marquis is susceptible to most of them.  $F_2$  seedling plants inoculated with a form of rust to which Kanred was immune and Marquis susceptible proved either resistant or susceptible. Families, homozygous for spring habit of growth and for resistance to all the forms of rust to which Kanred was resistant, were obtained in the  $F_3$ . Here a single factor apparently determined the reaction to several biologic forms, to 11 of which Kanred is immune.

Of the 37 known biologic forms of stem rust 21 were found in the area where hard red spring wheats are grown.<sup>4</sup> The most recent study in disease-resistance breeding at the Minnesota station shows the possibility of synthetically producing a desirable *vulgare* variety of wheat that will be resistant to all of these biologic forms (17). This is to be accomplished by means of several crosses and double crosses between varieties having the desired resistance and those having the necessary bread-wheat qualities for yield, milling, baking, etc.

In the present investigation use was made of a cross between two varieties of durum wheat which react reciprocally to two biologic forms of rust, each being resistant to the form to which the other is susceptible. It was hoped that some  $F_2$  families would prove resistant to both forms of rust. In addition to a study of the genetic factors concerned with resistance the relation of seed color to rust resistance was also considered. That resistance to several biologic forms is governed by a single factor, in the Kanred  $\times$  Marquis cross, indicates the importance of learning as much as possible about the inheritance of resistance to various rust forms in different crosses.

#### MATERIALS AND METHODS

The  $F_2$  families<sup>5</sup> of two *T. durum* crosses, Kubanka 8 (C. I. 4063)  $\times$  Pentad (C. I. 3322) and Mindum (Minn. No. 470)  $\times$  Pentad, were used. Kubanka No. 8 is a white-seeded selection from common Kubanka (C. I. 1440). Pentad is a red-seeded variety selected at the North Dakota station. Mindum is a white-seeded selection of the Arnautka type. Seedling plants from each  $F_2$  family were inoculated in the greenhouse with two biologic forms of *P. graminis tritici*. The crosses were made in 1920, and the  $F_1$  generation was grown in the greenhouse at Washington, D. C., during the winter of 1920-21. The  $F_2$  generation was grown in the plant-breeding nursery at University Farm, St. Paul, Minn., during the 1921 season, the plants being harvested individually.

<sup>4</sup> Unpublished results of Stakman and Levine.

<sup>5</sup> The term " $F_2$  family" is used in this article for the seedling plants grown from the seeds of a single  $F_2$  plant.

The material was a part of that which was being used for the purpose of obtaining resistant wheat for growing in the Northwest. This problem was being cooperatively investigated by the Sections of Plant Breeding and Plant Pathology of the Minnesota Agricultural Experiment Station, and the Office of Cereal Investigations of the United States Department of Agriculture. The individually harvested  $F_2$  plants were furnished to the present writers. The biologic forms of rust were some of those which were being studied by M. N. Levine.

Plants of each  $F_2$  family were grown in 4-inch pots in the greenhouse, one family in each pot. All pots of soil were steam sterilized immediately before use and the seed was sown at a uniform depth. A large proportion of the hybrid seed as well as of the parental seed was infected with *Helminthosporium* and consequently the number of plants per pot varied between 8 and 20. The seedlings were grown in a seedling section of the greenhouse in which no rust-infected plants were kept. When they were about 3 inches high they were inoculated with fresh urediniospores of one of the two forms used. Only one rust form was worked with at one time. The pots were then placed in an incubation chamber for 40 to 48 hours with a number of control pots of the parental varieties inoculated in the same way. The two forms of rust were kept on well-separated benches to avoid accidental contamination of one with the other. An attempt was made to keep the temperature, moisture, and light condition the same for all plants. The methods used were essentially the same as those described by Stakman and Piemeisel (29).

Notes on the character of infection with both forms were taken from 15 to 19 days after the date of inoculation. The amount and rapidity of rust development was found to vary in response to environmental conditions. However, the character of infection was found fairly constant within certain limits, regardless of external influences.

The  $F_2$  seedling plants were inoculated in sets of about 25 to 40 pots each. The total interval during which results were being recorded for the reaction to any one biologic form was approximately two weeks. In addition to running control plants of the parental varieties with each set of hybrid plants, one or more complete sets of "differential" varieties were inoculated during work with each form. In this way a complete control was obtained on the identity of the form being used. The differential varieties used were as follows: Little Club (C. I. 4066); Marquis; Kanred; Kota (C. I. 5878); Arnautka (C. I. 4072); Kubanka (C. I. 2094); Mindum (Minn. No. 470); Acme; Einkorn (C. I. 2433); Vernal emmer (Minn. No. 1165); and Khapli emmer (C. I. 4013).

The percentage of infected plants obtained, on the average, was very satisfactory. Of a total of more than 10,321 plants which were inoculated 796 showed no infection.

In recording the types of infection the symbols prepared by Stakman and Levine (25a) in their work on biologic specialization were used. These classes of host reaction and of corresponding types of infection resulting from inoculation of seedling wheat plants with spores of *Puccinia graminis tritici* are as follows:

Classes of host reaction	Types of infection <sup>6</sup>
Immune . . . . .	{ 0. No uredinia developed but definite hypersensitive areas present.
Very resistant . . . . .	{ 1. Uredinia minute and isolated; surrounded by sharp, continuous, hypersensitive areas; hypersensitive areas lacking uredinia also may be present.
Moderately resistant . . . . .	{ 2. Uredinia isolated and small to medium in size; hypersensitiveness present in the form of necrotic areas or circles; uredinia often surrounded by green islands.
Moderately susceptible (apparently resistant in the field) . . . . .	{ 3. Uredinia midsize; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.
Very susceptible . . . . .	{ 4. Uredinia large or varying from midsize to large, numerous and confluent; true hypersensitiveness entirely absent; chlorosis seldom present.
Resistant to moderately susceptible (apparently resistant in the field) <sup>7</sup> . . . . .	{ x - Uredinia very variable; apparently includes two or more of the 0, 1, 2, and 3 types of infection on the same leaf blade; no mechanical separation possible; on reinoculation one type of uredinia may produce another; infection ill-defined.
Moderately susceptible to very susceptible (probably susceptible in the field) . . . . .	{ x + Uredinia very variable; apparently including combinations of one or more of the types 0, 1, and 2, with type 4 on the same leaf blade; no mechanical separation is possible; on reinoculation one type of uredinia may produce a different type; infection ill-defined.

## SELECTION OF BIOLOGIC FORMS

In October, 1921, about 15 of the 37 known biologic forms were available. The reaction of Mindum to these forms was known but Kubanka No. 8 and Pentad had not been tested. It was hoped that among the 15 forms some could be found that would react reciprocally on the parents of the crosses to be tested. Extensive studies disclosed that Forms I and XXXIV reacted reciprocally on Mindum and Pentad. However, similar success was not achieved with respect to Kubanka No. 8 and Pentad. Therefore, only one form, No. XXXIV, was used for the Kubanka 8 × Pentad cross.

The first rust form, designated as Form I, was collected at St. Paul, Minn., in September, 1915. When used for the present study it had been cultured through approximately 90 urediniospore generations and there was no indication of a change in its parasitic capabilities. Mindum is decidedly resistant to this form, whereas Pentad and Kubanka No. 8 are normally susceptible.

The second form, known as Form XXXIV, was collected by G. F. Puttick at Potchefstroom, South Africa, in April, 1921. When it was received at the Minnesota station it was evidently mixed with one other form. The separation of these forms was not completed until December, 1921. Since then more than seven generations have been produced without any indication of a change in the constancy of the form. In contrast to its reactions to Form I, Mindum is quite susceptible to Form XXXIV and Pentad is resistant. Kubanka No. 8 is susceptible to this form.

<sup>6</sup> These descriptions of types of infection are based on those given by Stakman and Levine (25a).

<sup>7</sup> Unpublished results of cooperative rust breeding work carried on at the Minnesota station.

## ACCURACY OF THE RESULTS

In estimating the accuracy of the types of infection that appeared on the hybrids the behavior of the rust on pure varieties was taken as a criterion of the average amount of variation that occurred. In Table I the results of inoculations made with Form I on February 10 and 20 are given for a number of varieties, including Mindum and Pentad. In several cases, as those of Little Club and of Khapli, the results from the two periods are practically identical. In the majority of cases, however, the rust development from the inoculations of February 10 was weaker than from those inoculations made February 20. For example, einkorn had type 3 infections from the first inoculation but type 4 from the later inoculation. Similarly, with Kota, the February 10 inoculations produced type 3 infections whereas type 4 resulted from the February 20 inoculations.

TABLE I.—A comparison of the types of infection produced by biologic Form I on several varieties of wheat at two different periods

Group and variety.	Date inoculated.	Rust development.	Number of plants apparently not infected.	Types of infection.							Total of infected plants.
				o	1	2	X-	3	X+	4	
Common wheats:											
Little Club.....	Feb.	10	Days 17	.....	.....	.....	.....	.....	.....	24	24
		20	16	.....	.....	.....	.....	.....	.....	13	13
Marquis.....		10	17	.....	.....	.....	.....	.....	.....	12	12
		20	16	2	.....	.....	.....	.....	.....	12	14
Kanred.....		10	17	14	.....	.....	.....	.....	.....	.....	14
		20	16	10	.....	.....	.....	.....	.....	1	11
Kota.....		10	17	.....	.....	.....	.....	14	.....	.....	14
		20	16	.....	.....	.....	.....	.....	.....	15	15
Durum wheats:											
Arnautka, C. I. 1493.		10	17	.....	11	.....	.....	1	.....	3	15
		20	16	.....	9	.....	.....	.....	.....	4	13
Kubanka, C. I. 4063.		10	17	5	.....	.....	.....	6	.....	2	13
		20	16	3	.....	.....	.....	.....	.....	12	15
Acme.....		10	17	2	.....	.....	.....	11	.....	2	15
		20	16	.....	.....	.....	.....	.....	.....	11	11
Mindum.....		10	17	1	19	8	.....	.....	.....	.....	28
		20	16	.....	30	.....	.....	.....	.....	1	31
Pentad.....		10	17	4	4	3	.....	10	1	2	24
		20	16	.....	.....	1	.....	3	.....	8	12
Einkorn.....		10	17	.....	.....	.....	.....	.....	14	.....	14
		20	16	.....	.....	.....	.....	.....	.....	.....	14
Emmers.											
Vernal.....		10	17	1	12	.....	.....	.....	.....	.....	13
		20	16	3	11	.....	.....	.....	.....	.....	.....
Khapli.....		10	17	.....	.....	13	.....	.....	.....	.....	13
		20	16	.....	.....	14	.....	.....	.....	.....	.....

The appearance of a type 4 infection on 1 out of 11 Kanred plants inoculated February 20 and similarly on 1 out of 31 Mindum plants, indicated accidental infection from some other form of rust, or impurity in the seed due to mechanical mixture or to natural crossing. The

Arnautka seed was known to be impure. This explains why 8 out of the 28 plants inoculated did not appear to be immune. Hayes (14) and Hayes and Garber (15, p. 35-36, 77-78) have reported from 2 to 3 per cent of natural crossing in wheat grown at St. Paul, Minn. A rather large difference is shown between the inoculations of February 10 and February 20 on Pentad. This demonstrates the value of running several pots<sup>8</sup> of the parental varieties with each set of hybrid families.

From the foregoing discussion it should not be concluded that the variations were so great as to obscure the results. The reaction of the  $F_3$  plants to the different forms of rust was constant within certain limits and the fact that the plants which were tested were of the third hybrid generation, and consisted of families each arising from a single  $F_2$  plant, makes the interpretation of the various reactions a fairly definite matter.

#### BASIS FOR INTERPRETING RESULTS

As the determination of the genetic nature of resistance was the chief object of this investigation, the results obtained were interpreted on the basis of the reactions of the parental varieties to the forms of rust used. The  $F_3$  families were placed in five classes with reference to reaction to the different biologic forms, viz, resistant, near-resistant, heterozygous, near-susceptible, and susceptible. In the work with Form I the class designations "immune" and "near-immune" were used instead of "resistant" and "near-resistant" owing to the nature of the Mindum reaction.

$F_3$  families showing resistance similar to that of the resistant parent were termed resistant. Similarly, the  $F_3$  families showing susceptibility equivalent to that of the susceptible parent were classed as susceptible. Those  $F_3$  families having 9 or more apparently resistant plants and one plant showing susceptibility were classed as near-resistant. Likewise  $F_3$  families with 9 or more plants showing susceptibility and one plant appearing resistant were placed as near-susceptible.

It is probable that some of the  $F_3$  families placed in the near-resistant or near-susceptible class were in reality heterozygous. It is also probable that others placed in these classes were resistant or susceptible, respectively. Owing to the likelihood of the occurrence of certain errors, which have been discussed in a previous section of this paper, it was not possible to determine the exact genetic nature of the "near-resistant" and "near-susceptible"  $F_3$  families. It was best, therefore, to leave these doubtful  $F_3$  families in classes which by their names indicate their character.

All  $F_3$  families not falling in one of the preceding four classes were termed heterozygous. The heterozygosity of various  $F_3$  families varied from a preponderance of apparently susceptible plants to a preponderance of plants showing resistance. Of the five different classes the resistant class is of greatest importance, as it represents the  $F_3$  families which appeared homozygous for resistance.

<sup>8</sup> The word "pot" is used throughout this article to denote the group of plants of a pure line parent variety growing in a single pot, such a group being comparable to a group of  $F_3$  plants (an  $F_3$  family) likewise growing in one pot.

## EXPERIMENTAL RESULTS

## THE SEED COLOR RATIO

The inheritance of seed color in wheat has been studied for a number of years. Ratios of 3:1, 15:1, and 63:1 of red-seeded and white-seeded plants, respectively, have been obtained in the  $F_2$  of crosses between red-seeded and white-seeded wheats (3, 18, 19, 11). Apparently there may be from one to three factors for red seed color present in different wheats. When one or more of the factors for red are present red seed results.

The results obtained in the present study are given in Table II. Of 364  $F_2$  plants of the Kubanka No. 8  $\times$  Pentad cross that were classified, 295 were red-seeded like the Pentad parent and 69 were white-seeded like the Kubanka No. 8 parent. The observed ratio was 3.21:0.79 with red seed color dominant. There were 599  $F_2$  plants of the Mindum  $\times$  Pentad crosses classified. The red-seeded totaled 449 and the white-seeded 150, the ratio being almost exactly 3:1 with red seed color dominant. In both cases the presence of a single factor for seed color is indicated.

TABLE II.—Classification of  $F_2$  families on the basis of seed color

Cross.	Family numbers of $F_2$ .	No. of $F_2$ families.		
		Red-seeded.	White-seeded.	Total.
Kubanka No. 8 $\times$ Pentad.....	774 to 792	295	69	364
Mindum $\times$ Pentad.....	793 to 810	247	82	329
Mindum $\times$ Pentad.....	644 to 680	202	68	270

THE  $F_2$  SEED COLOR IN RELATION TO THE RUST REACTION OF THE PROGENYTABLE III.—The relation of seed color to the reaction of the  $F_3$  generation of two durum crosses to two biologic forms of *P. graminis tritici*

Parents of F <sub>2</sub> families.		Biologic form used.	Reaction of F <sub>2</sub> families.					
Cross.	Seed color.		Classes of infection.					Total number of families.
			Resistant or immune	Near-resistant or near-immune.	Heterozygous.	Near-susceptible.	Susceptible.	
Kubanka No.8×Pentad	Red ...	XXXIV.	8	4	70	15	104	201
Do.....	White ..	XXXIV.	1	1	14	5	24	45
Mindum×Pentad.....	Red ...	I.....	23	7	39	6	6	81
Do.....	White..	I.....	21	6	43	4	11	85
Do.....	Red ...	XXXIV.	9	2	48	10	11	80
Do.....	White..	XXXIV.	5	4	20	1	0	30

Table III summarizes separately the results for the  $F_3$  families arising from red-seeded  $F_2$  parents and for those from white-seeded  $F_2$  parents. The Kubanka 8  $\times$  Pentad  $F_2$  families reacted with Form XXXIV as follows: Of those from red-seeded parents, 8 appeared to be resistant, 4 near-

resistant, 70 heterozygous, 15 near-susceptible, and 104 susceptible; of those from white-seeded parents, 1 was classed as resistant, 1 near-resistant, 14 heterozygous, 5 near-susceptible, and 24 susceptible. A brief comparison of these two sets of figures reveals their similarity, that is to say, no correlation of seed color with resistance or susceptibility to rust was indicated.

Mindum  $\times$  Pentad  $F_2$  families when inoculated with Form I gave the following results: Of those from red-seeded parents, 23 were classed as immune, 7 near-immune, 39 heterozygous, 6 near-susceptible, and 6 susceptible; of those from white-seeded parents, 21 were placed as immune, 6 near-immune, 43 heterozygous, 4 near-susceptible, and 11 susceptible. Here again the distribution of families from red seed was similar to that of the progeny of white-seeded plants. A lack of correlation of seed color with resistance or susceptibility to rust was evident.

Mindum  $\times$  Pentad  $F_2$  families when tested with Form XXXIV reacted in the following ways: Of those from red-seeded parents, 9 appeared resistant, 2 near-resistant, 48 heterozygous, 10 near-susceptible, and 11 susceptible; of those from white-seeded parents, 5 were classed as resistant, 4 near-resistant, 20 heterozygous, 1 near-susceptible, and none susceptible. A comparison of the distribution of the progeny of the red-seeded parents with that of families arising from white-seeded parents shows differences in the near-susceptible and susceptible classes. These, however, can not be given much weight owing both to the small number of families classified and to the fact that Mindum, the white-seeded parent, was susceptible, whereas only 1 of the 30 families from white-seeded  $F_2$  was in the near-susceptible and susceptible classes. If anything, a negative correlation is indicated.

A summarization of the data concerning the mode of inheritance of rust resistance with respect to seed color shows that no correlation was found to exist between the two. It is important to note that each of the three sets of data shows several  $F_2$  families which were homozygous for the reaction of the Pentad parent and also homozygous for the seed color of Mindum or Kubanka No. 8 parents. Rust resistance and seed color appeared to be inherited independently.

#### RESULTS OF INOCULATIONS OF KUBANKA NO. 8 $\times$ PENTAD $F_2$ FAMILIES WITH BIOLOGIC FORM XXXIV.

The data obtained for Kubanka No. 8  $\times$  Pentad with Form XXXIV are summarized in Table IV. About 35 control pots of Kubanka No. 8 were inoculated. Of the 308 plants that were infected, 21, or about 7 per cent, had type 1 infections and 287 showed types ranging from x- to 4. In view of the fact that possible errors and variations may result in types of infection outside of the normal range, the type 1 infection on these 21 plants was regarded here as exceptional. The range of infection types on Kubanka No. 8 tested with Form XXXIV was therefore considered to be from x- to 4 (Pl. I, B). All Kubanka No. 8 pots with plants giving types of infection falling within this range were classified as susceptible. Those pots with 9 or more plants, one of which had a type 1 infection, were placed in the near-susceptible class. Pots having a greater percentage of plants with type 1 infections were classed as heterozygous. The distribution of the Kubanka No. 8 pots in the five classes of infection was 5 heterozygous, 5 near-susceptible, and 25 susceptible. There is no reason to believe that 5 of the parent control pots were actually heterozygous



but, rather, the method of classification shows the accuracy with which the determination of pure-line material could be made.

TABLE IV.—*The reaction of Kubanka No. 8 × Pentad and F<sub>2</sub> families to biologic Form XXXIV*

Parent varieties and F <sub>2</sub> families.	Class of infection.	Distribution of F <sub>2</sub> plants according to the type of infection shown.						Total of pots of parent varieties or F <sub>2</sub> families.	
		o	1	2	X—	3	X+		4
Kubanka No. 8	Heterozygous		15		10		6	14	5
Do.	Near-susceptible		6		5	6	14	15	5
Do.	Susceptible				56	1	24	136	25
Total			21		71	7	44	165	35
Pentad	Resistant	4	270		30				30
Do.	Near-resistant		36		4		4		4
Do.	Heterozygous		12		2		7		2
Total		4	318		36		11		36
F <sub>2</sub>	Resistant		70						9
Do.	Near-resistant		52		10				5
Do.	Heterozygous		297		183		91	256	84
Do.	Near-susceptible		5		30	3	31	112	20
Do.	Susceptible		1		108	13	114	1,025	128
Total			425		331	16	236	1,393	246

The legitimacy of the foregoing method of analysis will become evident if a brief study is made of the type of data obtained and the object of the work. To make possible a classification which would form an adequate basis for the comparison of the reactions of the F<sub>2</sub> hybrids with those of the parental varieties it was necessary to establish a range for the types of infection normal for each parent. It might appear that the total distribution of types of infection of a parental variety should be considered its normal range, and the hybrid reactions judged accordingly. This would throw all the pots of each parent into a single class, viz, with Kubanka No. 8 there would be 35 pots in the susceptible class, which would be the same as ignoring the possibility of any error in the results. Obviously that would lead to inaccuracies in classifying the hybrids, for it is well established that even with carefully controlled conditions a certain amount of error is unavoidable. The method used has on the contrary much in its favor, for it tends to throw all doubtful F<sub>2</sub> families into the near-susceptible, heterozygous, or near-resistant classes.

Thirty-six control pots of Pentad were inoculated. Of the 369 plants that were infected, all but 11 ranged from o to X— in type of infection. These 11 showed infection of the X+ type. Classification of the pots on the same basis as was used in the case of Kubanka No. 8 resulted in 30 being placed as resistant, 4 near-resistant, and 2 heterozygous (Pl. I, A).

The use of these terms to designate the different classes of infection is relative. It is convenient to call one parent susceptible and the other resistant regardless of whether the susceptibility or resistance is partial or complete. For the purpose of studying the genetics of the inheritance

of the rust reaction it is immaterial just what the classes are called. In the present cross, the Kubanka No. 8 parent is only partially susceptible but the Pentad parent is quite resistant. It thus happens that the ranges for the two parent varieties overlap somewhat at the x- type of infection.

A total of 246  $F_3$  families were tested. The number of  $F_3$  plants that showed infection was 2,401. The distribution of  $F_3$  plants according to their types of infection and to the susceptible classes of the various families to which they belonged is given in Table IV. The 246  $F_3$  families were classified as 9 resistant, 5 near-resistant, 84 heterozygous, 20 near-susceptible, and 128 susceptible (Pl. I, C-E). Then, one-sixth of the pots of Pentad, the resistant parent, were classed other than resistant. Considering the  $F_3$  on this basis, there were 9 plus 1.5, or 10.5 families which were as resistant as the resistant parent. The ratio of this number to that of the remaining families is 1:22.4. The presence of 2 factors for rust reaction fairly satisfactorily explains the results. There was no evidence that either susceptibility or resistance was dominant, for the  $F_3$  plants in the heterozygous class occurred in approximately equal numbers on either side of the x- type.

RESULTS OF INOCULATIONS OF MINDUM  $\times$  PENTAD  $F_3$  FAMILIES WITH BIOLOGIC FORM I

The data obtained with Form I are summarized in Table V. Twenty-three control pots of each of the parental varieties were inoculated. Of the 274 Mindum plants that were infected, 269 had 0 and 1 types of infection with a killing of the leaf tips in most cases, and 5 had type 4 infections. These 5 plants clearly were either not Mindum or else they had been infected by chance spores of some other form. Accidental infection is almost unavoidable where many rust forms are being worked with but fortunately it is of infrequent occurrence and usually can be detected.

TABLE V.—The reaction of the  $F_3$  of Mindum  $\times$  Pentad to biologic Form I

Parent varieties or $F_3$ families.	Class of infection.	Distribution of $F_3$ plants according to the type of infection shown.							Total pots of parent varieties or $F_3$ families.
		0	1	2	X-	3	X+	4	
Pentad.....	Heterozygous.....	13	4	.....	9	1	5	.....	4
Do.....	Near-susceptible....	1	2	.....	6	3	10	.....	3
Do.....	Susceptible.....	.....	.....	.....	10	13	14	76	16
Total.....	.....	14	6	.....	25	17	29	76	23
Mindum.....	Immune.....	236	12	.....	.....	.....	.....	.....	20
Do.....	Near-immune.....	15	.....	.....	.....	.....	.....	2	2
Do.....	Heterozygous.....	6	.....	.....	.....	.....	.....	3	1
Total.....	.....	257	12	.....	.....	.....	.....	5	23
$F_3$ .....	Immune.....	603	.....	.....	.....	.....	.....	.....	44
Do.....	Near-immune.....	158	.....	.....	.....	7	1	5	13
Do.....	Heterozygous.....	723	9	2	16	60	22	190	82
Do.....	Near-susceptible....	10	3	.....	10	22	15	68	10
Do.....	Susceptible.....	.....	.....	.....	6	72	2	165	17
Total.....	.....	1,494	12	2	32	161	40	428	166

The range of infection for Mindum was considered to include 0 and 1 types of infection. In the immune class, 236 of the 248 plants had 0 type infections, hence the immunity of Mindum to Form I is evident. The pots of Mindum were classified as 20 immune, 2 near-immune, and 1 heterozygous (Pl. 2).

In the 23 control pots of Pentad 167 plants were infected. Of these, 147 had types of infection ranging from x- to 4, and 20 showed types 0 and 1. Leaving these 20 plants out of consideration, the range of the type of infection was from x- to 4, as was the case with Kubanka No. 8 when inoculated with Form XXXIV. At first glance it would seem that 20 is a rather large number of plants to leave out of consideration in a population of 167. It is not as significant as it appears to be, however, for many of the plants with 0 type infections showed only a few vague necrotic flecks which were not clearly distinguishable from similar effects sometimes produced on seedlings as a result of environmental conditions. The classification of Pentad pots gave 16 as susceptible, 3 near-susceptible, and 4 heterozygous (Pl. 2).

A total of 166  $F_3$  families were tested. The number of  $F_3$  plants inoculated was 2,340. Of these 2,169 showed infection. The distribution of  $F_3$  plants according to their types of infection and to the classes of the various families to which they belonged is given in Table V. The 166  $F_3$  families were classified as 44 immune, 13 near-immune, 82 heterozygous, 10 near-susceptible, and 17 susceptible (Pl. 2).

A larger proportion of families was classified as immune and near-immune than as susceptible and near-susceptible. This was to be expected from the progeny of the parents, one of which was immune and the other only moderately susceptible. Approximately one-eighth of the pots of Mindum, the immune parent, fell outside the immune class. The  $F_3$  progeny, considered on this basis, shows 44 plus 5.5, or a total of 49.5 families which appeared to be as immune as the immune parent. The ratio of 49.5 to 166, which is the total number of families classified, would seem to indicate the presence of a single differential factor. The variable results obtained with the Pentad parent prevent a more detailed analysis.

The distribution of plants in the heterozygous class with respect to the various types of infection shows a total of 734 with 0, 1, and 2 types of infection (723 being of the 0 type) and 288 with types ranging from x- to 4. The dominance of immunity is evident.

#### RESULTS OF INOCULATIONS OF MINDUM×PENTAD $F_3$ FAMILIES WITH BIOLOGIC FORM XXXIV

The results obtained are summarized in Table VI. Thirty-three control pots of each of the parental varieties were inoculated. The reactions of Mindum and Pentad to Form 34 were the reciprocals of their reactions to Form I. Of the 375 Mindum plants that showed infection all but 9 had x+ and 4 types of infection. The remaining 9 plants had x- and 3 types of infection. Mindum is here the susceptible parent with a range of types of infection which is narrower than that of the susceptible parents in the work previously discussed. The pots of Mindum were classified as 2 heterozygous, 4 near-susceptible, and 27 susceptible (Pl. 3).

TABLE VI.—The reaction of the parent varieties and  $F_2$  families of Mindum  $\times$  Pentad on biologic Form XXXIV.

Parent varieties or $F_2$ families.	Class of infection.	Distribution of $F_2$ plants according to the type of infection shown.							Total pots of parent varieties or $F_2$ families.
		0	1	2	X—	3	X+	4	
Mindum . . . . .	Heterozygous . . . . .				3	2	2	14	2
Do . . . . .	Near-susceptible . . . . .				1	3	7	31	4
Do . . . . .	Susceptible . . . . .						39	273	27
Total . . . . .					4	5	48	318	33
Pentad . . . . .	Resistant . . . . .	37	115	3	131	2			26
Do . . . . .	Near-resistant . . . . .	3	18		24		5		5
Do . . . . .	Heterozygous . . . . .		2		9	3	4	1	2
Total . . . . .		40	135	3	164	5	9	1	33
$F_2$ . . . . .	Resistant . . . . .	38	53	8	95				14
Do . . . . .	Near-resistant . . . . .	12	29	1	53		8		6
Do . . . . .	Heterozygous . . . . .	38	48	8	400	144	232	139	68
Do . . . . .	Near-susceptible . . . . .				8	4	47	90	11
Do . . . . .	Susceptible . . . . .					1	39	109	11
Total . . . . .		88	130	17	556	149	326	338	110

Infection appeared on 357 of the inoculated Pentad plants. Of these 9 reacted with x+ type infections and 1 gave a 4 type. The other 347 Pentad plants gave a range of infection types from 0 to 3. Only 5 plants showed type 3 infections, therefore this type would appear not to belong to the range for the resistant parent. However, the type 3 infection is much more like the x— type than like the x+. In fact, experiments indicate that a variety of which the seedling plants give type 3 infection in the greenhouse is resistant in the field, whereas varieties showing x+ in the greenhouse probably are not resistant in the field. Consequently, the 3 type infection may well be included in the range of the Pentad parent. Classification of Pentad pots gave 26 resistant, 5 near-resistant and 2 heterozygous (Pl. 3).

A comparison of the results obtained for Pentad in the study with the Kubanka No. 8  $\times$  Pentad cross with those obtained here reveals considerable differences in the number of plants showing the various types of infection. The two series of inoculations were made at different periods, and consequently the conditions were not comparable. This strikingly brings out the fact that the value of the data taken on the hybrid material lays in the direct comparison made with the results from pots of parental plants grown under identical conditions. Reactions which, under one set of circumstances, would place an  $F_2$  family in the near-resistant class might, under other circumstances, cause it to be classed as heterozygous, depending on the parental reactions at each period.

It was noticeable that both parent and hybrid plants showed an abundance of hypersensitiveness to Form XXXIV. This probably accounts in great measure for the appearance of such large numbers of x— and x+ types of infection. Sharp hypersensitive areas occurred around a large proportion of the uredinia of types 3 and 4 infections. Usually type 4 shows

very little chlorosis and no necrosis. Here, however, about 50 per cent of the type 4 uredinia showed such a decided necrotic bordering that the advisability of creating a special class for this type of infection was considered. Eventually it was concluded that the establishment of an additional type would possibly lead to some confusion without really proving of value, as it was apparent that plants infected in this manner were susceptible, on account of the development of numerous large uredinia.

A total of 110  $F_3$  families were tested. There were 1,711  $F_3$  plants inoculated, of which 1,604 showed infection. The distribution of  $F_3$  plants is summarized in Table VI. Classification of the 110  $F_3$  families gave 14 as resistant, 6 near-resistant, 68 heterozygous, 11 near-susceptible, and 11 susceptible (Pl. 3). Since between a third and a fourth of the Pentad pots were placed outside of the resistant class, it may be considered that 14 plus 4 or 18  $F_3$  families were as resistant as the resistant parent. This number of resistant families is too small to indicate definitely the presence of a single factor, and it is much too large to make a two-factor hypothesis feasible. The single factor is the more plausible explanation.

If the near-resistant and resistant classes are grouped together, and similarly, the near-susceptible and susceptible classes, a fairly satisfactory 1:2:1 ratio is obtained. The presence of a single factor would appear probable in this case. The results do not make possible a more exact genetic analysis. Examination of the distribution of  $F_3$  plants in the heterozygous class showed a lack of dominance for either susceptibility or resistance.

#### COMBINATIONS OF SUSCEPTIBILITY AND RESISTANCE OF MINDUM $\times$ PENTAD $F_3$ FAMILIES TO FORM I AND FORM XXXIV

Table VII correlates the results obtained with Form I and Form XXXIV for those families which were inoculated with both forms. Various combinations of susceptibility, resistance, and heterozygosity to the two biologic forms appear. Six  $F_3$  families were definitely resistant to Form XXXIV and immune from Form I as, for example, 809-21, 805-16, and 796-4 (Pl. 4, A, B, C). Two families were quite susceptible to both forms, as 804-7 (Pl. 4, E). Other families were resistant to or immune from one form and susceptible to the other. Still other  $F_3$  families were resistant or susceptible to one form and heterozygous to the other, as 806-24 (Pl. 4, D). Many families were heterozygous to both forms. There were also combinations of near-resistance and near-susceptibility, as 809-8 (Pl. 4, F).

TABLE VII.—The classified reaction of Mindum  $\times$  Pentad  $F_3$  families to biologic Forms I and XXXIV

Reaction to Form XXXIV.	Reaction to Form I.					Total.
	Immune.	Near-immune.	Heterozygous.	Near-susceptible.	Susceptible.	
Resistant.....	6	0	8	0	0	14
Near-resistant.....	1	0	2	1	1	5
Heterozygous.....	14	7	36	3	7	67
Near-susceptible.....	3	1	5	1	1	11
Susceptible.....	3	1	3	1	2	10
Total.....	27	9	54	6	11	107

The most significant feature of Table VII is that six  $F_2$  hybrid families are shown to have been definitely resistant to both biologic Forms I and XXXIV. Considering the reaction of the parental varieties to these biologic forms, it is very likely that at least one of the two near-resistant families was likewise homozygous in its reaction. On this basis it would seem that two main differential factors may explain the results obtained. These factors, if present, are inherited independently, which makes possible the combining of resistance to both forms in a single variety.

THE REACTION OF THE MINDUM  $\times$  PENTAD  $F_2$  FAMILIES FROM WHITE-SEEDED PARENTS TO FORM I AND FORM XXXIV

Table VIII correlates the results obtained for  $F_2$  families from white-seeded parents when inoculated with Forms I and XXXIV. Out of a total of 34 families 2 were resistant to Form XXXIV and immune from Form I. The indications of 3:1 ratios for Forms I and XXXIV are here substantiated in a very close 15:1 ratio.

TABLE VIII.—The classified reaction of the progeny of Mindum  $\times$  Pentad white-seeded  $F_2$  plants to biologic Forms I and XXXIV

Reaction to Form XXXIV.	Reaction to Form I.					Total.
	Immune.	Near-immune.	Heterozygous.	Near-susceptible.	Susceptible.	
Resistant.....	2	0	3	0	0	5
Near-resistant.....	1	0	2	0	2	5
Heterozygous.....	3	2	11	1	2	19
Near-susceptible.....	0	0	0	0	1	1
Susceptible.....	0	0	0	0	0	0
Total.....	6	2	16	1	5	30

The object of giving special treatment to the  $F_2$  progeny of white-seeded  $F_2$  plants is a recognition of the fact that the economic aspects of a problem warrant careful consideration. Pentad, although high in rust resistance, is a poor milling wheat. Owing to the difficulty of distinguishing the thrashed grain of one red durum from another, millers have come to discriminate against all red durums. There is consequently no immediate future for a new rust-resistant red durum, even though it be of good milling quality.

#### DISCUSSION

The study of the reaction of the  $F_2$  generation of the durum crosses Mindum  $\times$  Pentad and Kubanka No. 8  $\times$  Pentad to two biologic forms of *P. graminis tritici* has shown that all combinations of susceptibility and resistance to these forms may appear. The reaction of Kubanka No. 8  $\times$  Pentad  $F_2$  families to Form XXXIV indicated the presence of two factors with neither susceptibility nor resistance dominant. The results obtained from Mindum  $\times$  Pentad  $F_2$  families inoculated with Form I can be explained on the basis of a single main factor difference with immunity dominant. The data for the reaction of Mindum  $\times$  Penta

F<sub>3</sub> families to Form XXXIV gave some indication that a single factor was present, but dominance was not apparent. No evidence of a correlation between seed color and rust reaction was found.

As the action of each biologic form in general was fairly uniform on individual plants of the differential varieties and also on the plants of Kubanka No. 8, Mindum and Pentad and the F<sub>3</sub> families, barring the x- and x+ types of infection, it may be concluded that the urediniospores of each form probably were of the same genetic constitution. Therefore, the factors concerned with susceptibility and resistance must have been located in the plants themselves. Furthermore, as different biologic forms differ in their parasitizing capabilities on the same varieties of wheat, their genetic constitution can not be the same. It is probable, then, that factors governing susceptibility and resistance to different biologic forms used in this experiment are different in nature and are located in different chromosome pairs. The results of the present investigation show that when two varieties of wheat react reciprocally to two biologic forms of *P. graminis tritici*, one variety being resistant to one form and the other to the other form, it is possible by means of crossing these varieties to obtain progeny resistant to both forms of rust. These results furnish further evidence for the belief that eventually a variety of wheat can be produced which will be resistant to all biologic forms of this rust.

#### SUMMARY

(1) A study was made of the parasitic capabilities of two biologic forms of *Puccinia graminis tritici* on the F<sub>3</sub> progeny from crosses between three varieties of *Triticum durum*.

(2) Two of the parental varieties, Mindum and Pentad, reacted reciprocally to two of the biologic forms used.

(3) The constancy of the parasitic capabilities of the biologic forms had been determined previously and, as additional evidence, complete sets of differential varieties were inoculated at the commencement and at the completion of the work with each form. Form XXXIV attacked Kubanka No. 8 but had little effect on Pentad. Form I produced no uredinia on Mindum but developed vigorously on Pentad. On the other hand, Form XXXIV infected Mindum severely but developed weakly on Pentad.

(4) A separate set of 8 to 20 plants from each F<sub>3</sub> family was grown for use with each form of rust. Only first leaves were inoculated.

(5) With respect to the reaction of the F<sub>3</sub> families to each of the two biologic forms of rust there were some families that were as resistant as the resistant parent, some as susceptible as the susceptible parent, and others which were heterozygous.

(6) The results of using Form XXXIV on Kubanka 8 × Pentad F<sub>3</sub> families indicated the presence of two differential factors.

(7) A single main factor difference explained fairly satisfactorily the results obtained for Mindum × Pentad F<sub>3</sub> families inoculated with Form I.

(8) The results obtained when Mindum × Pentad F<sub>3</sub> families were infected with Form XXXIV, gave some indication of the presence of a single factor. The data would not permit, however, a definite genetic analysis.

(9) No relation was found between seed color and rust resistance.

(10) All combinations of susceptibility and resistance of individual Mindum × Pentad  $F_2$  families to Form I and Form XXXIV appeared. Out of a total of 110  $F_2$  families 6 were highly resistant to both forms of rust and 2 of these families were from white-seeded parents.

(11) With the varieties of wheat used, it was found possible to combine, in a single variety, resistance to two biologic forms of stem rust of wheat, when crosses were made between two varieties which reacted reciprocally to these rust forms.

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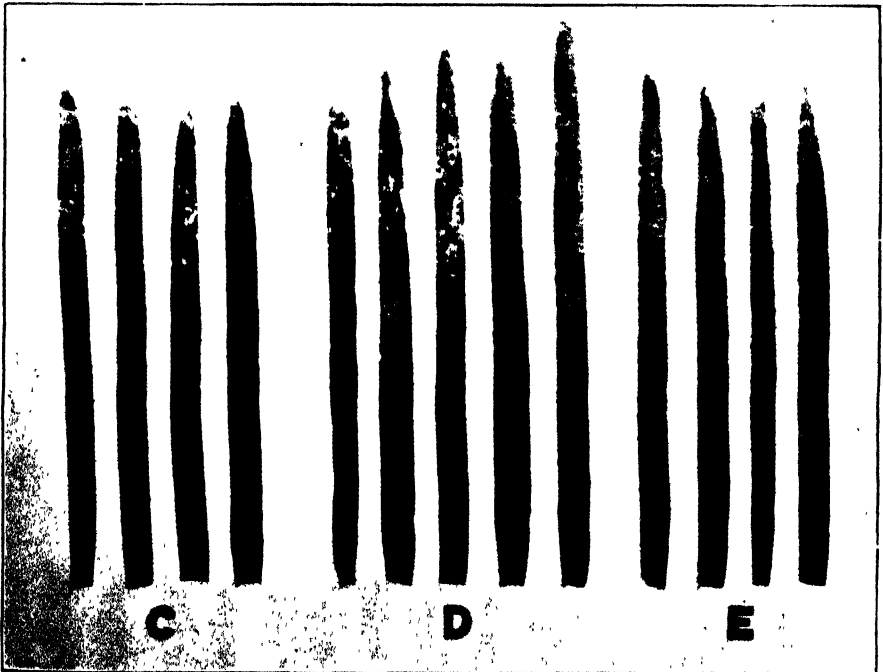
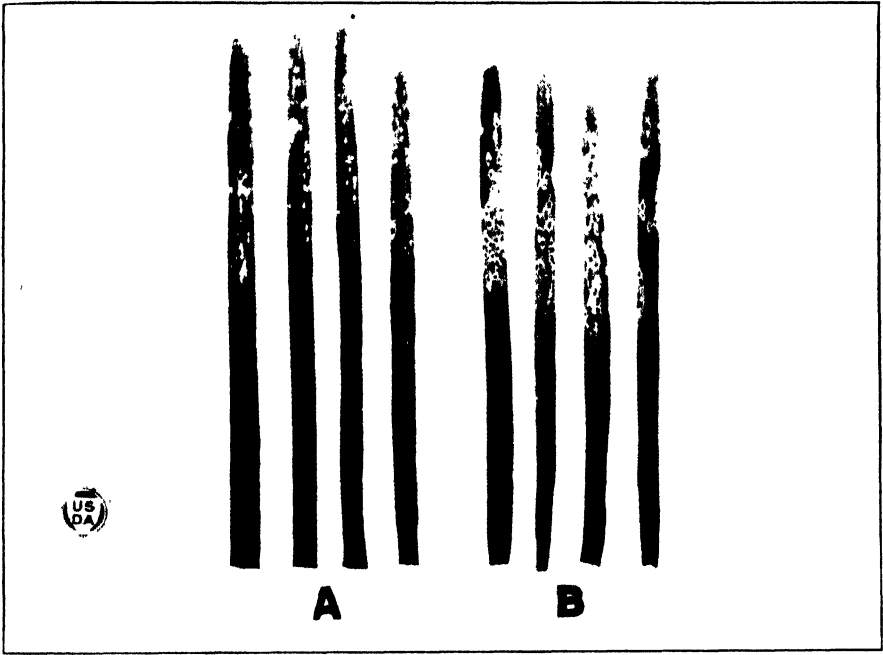
PLATE I

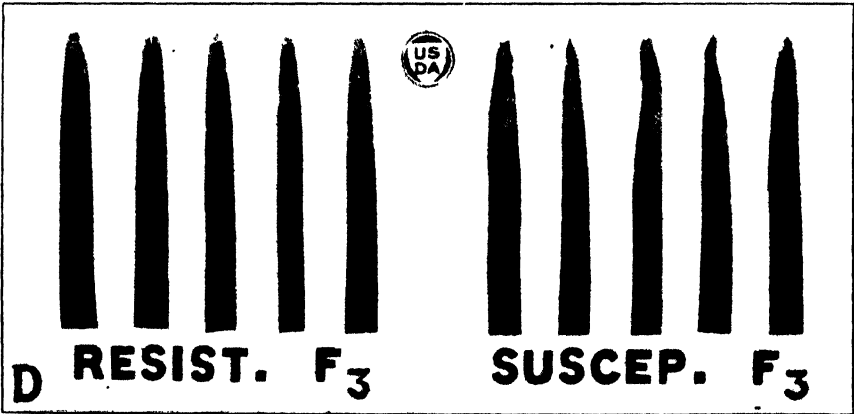
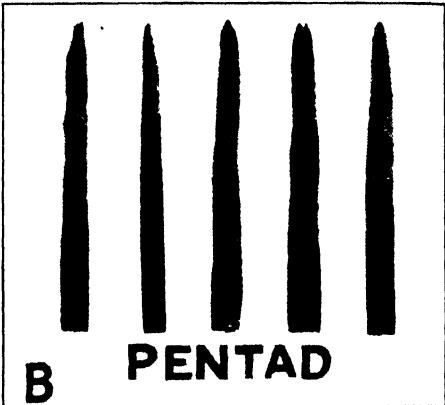
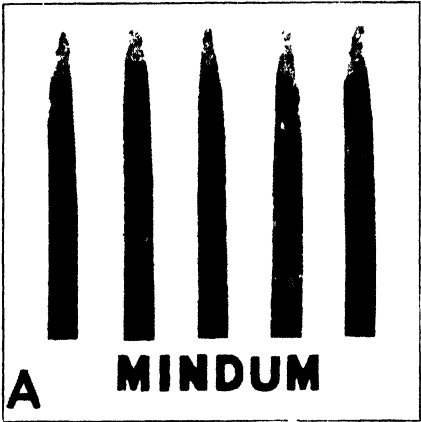
The reaction of Pentad, Kubanka No. 8, and  $F_3$  hybrids to biologic Form XXXIV.

A.—Pentad, the resistant parent, normally giving the o-type of infection with an occasional uredinium.

B.—Kubanka No. 8, the susceptible parent, giving numerous uredinia more or less surrounded by chlorotic areas.

C, D, E.—Typical  $F_3$  families that appeared resistant, heterozygous, and susceptible, respectively.





## PLATE 2

The reaction of Mindum, Pentad, and  $F_3$  hybrids to biologic Form I.

A.—Mindum is immune, normally giving a few hypersensitive flecks.

B.—Pentad is susceptible with types of infection varying from x- to 4.

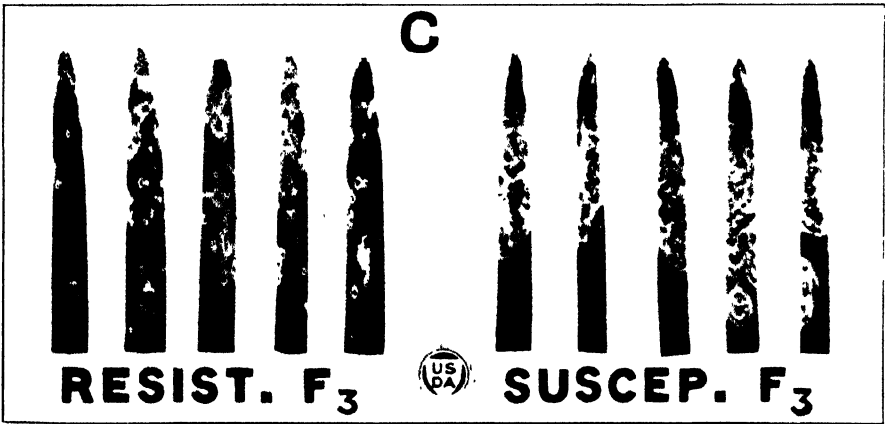
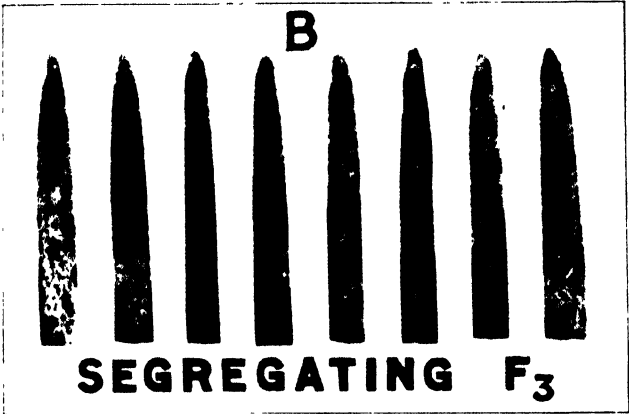
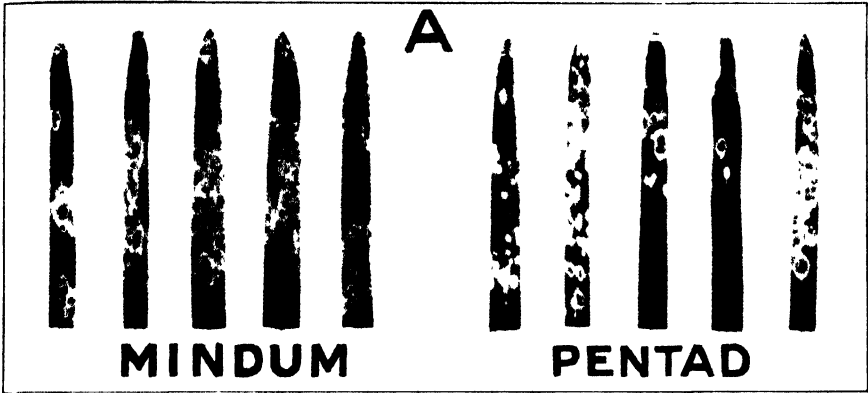
C, D.—The  $F_3$  families shown are, respectively: 802-6, which appeared heterozygous; 809-5, which was immune like Mindum; 804-17, which showed greater susceptibility than Pentad.

### PLATE 3

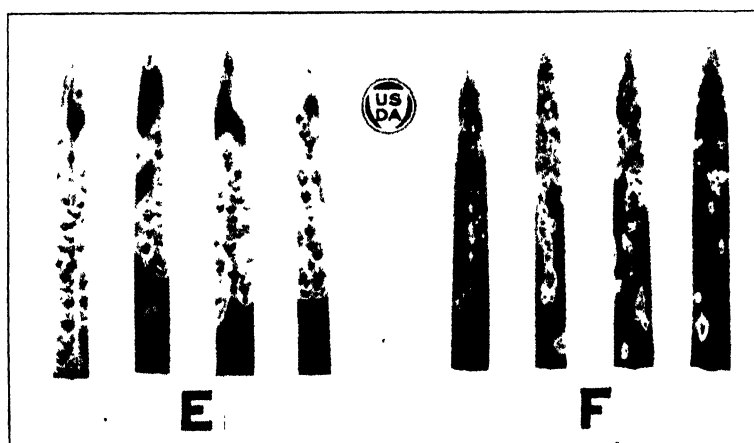
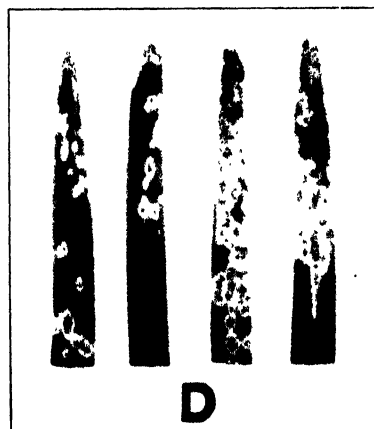
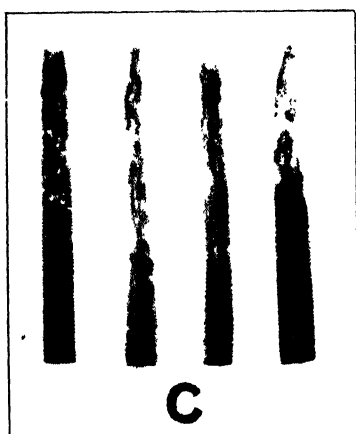
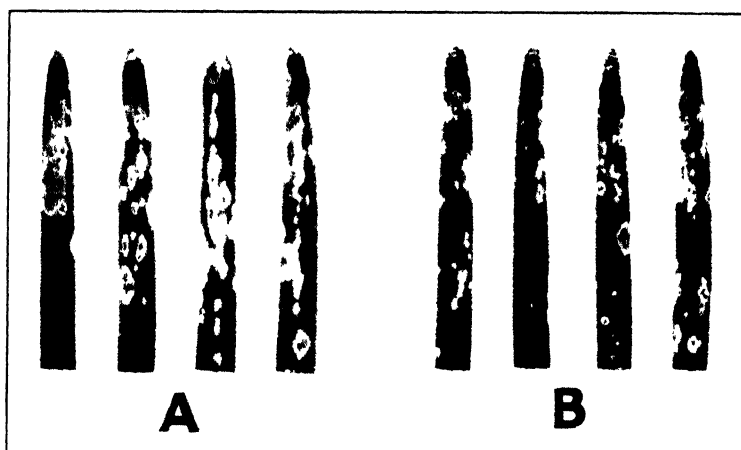
The reaction of Mindum, Pentad, and  $F_2$  hybrids to biologic Form XXXIV.

A.—Mindum, the susceptible parent, normally giving  $\times+$  and 4 types of infection, principally the latter; Pentad, the resistant parent, with a range of infection types from 0 to  $\times-$ .

B, C.—The  $F_2$  families shown are, respectively: 807-18, heterozygous; 805-16, as resistant as the resistant parent; 804-17, susceptible like Mindum.







#### PLATE 4

The reaction of certain Mindum  $\times$  Pentad  $F_3$  families to biologic Form XXXIV.

A, B.—Two  $F_3$  families (809-21 and 805-16, respectively) from white-seeded parents, immune from Form I and resistant to Form XXXIV.

C.—Family 796-4, from red-seeded parents and similar in reaction to A and B.

D.—Family 806-24, which was heterozygous to Form I and resistant to Form XXXIV.

E.—Family 804-7, which was highly susceptible to both forms of rust.

F.—Family 800-8, which fell into the near-susceptible class with Form I and in the near-resistant class with Form XXXIV.



# A STUDY OF RUST RESISTANCE IN A CROSS BETWEEN MARQUIS AND KOTA WHEATS<sup>1</sup>

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## INTRODUCTION

A knowledge of the mode of inheritance of certain plant characters is essential if the breeder is to outline his particular problem. Accordingly one of the first steps in the investigations carried on for the purpose of producing rust-resistant spring wheats has been to determine the genetic possibilities of attaining the desired end.

Several years ago it was a common belief that the rust organism changed its infection capabilities so rapidly as to seriously interfere with the production of rust-resistant wheat. The investigations of Stakman and his coworkers (6, 7, and 8)<sup>2</sup> at the Minnesota station have proved that the variable results which have been obtained under field conditions are due to numerous biologic forms of rust which can only be differentiated by their manner of infecting particular pure lines of wheat. This has led naturally to the survey to determine the prevalence of particular rust forms and to the use of these forms in breeding experiments. The method is to determine those wheat varieties which are resistant to particular rust forms and then by means of crosses to build up desirable rust-resistant varieties.

Kota (9) is the only rust-resistant spring common wheat which so far has been discovered, except for certain recently produced hybrids. Under humid conditions, however, Kota does not yield as well as Marquis and is seriously lacking in strength of straw.

The purpose of this paper is to present certain studies of a cross between Kota and Marquis which were carried on with the hope of solving the following questions:

1. Is the resistance of Kota to different biologic forms due to a single genetic factor, as in the case of Kanred?
2. Given Kota and Marquis, which react reciprocally to two biologic forms of rust, is it possible to produce from a cross of Kota and Marquis a variety resistant to both biologic forms?
3. To determine the possibility of using the reaction of  $F_3$  seedlings in the greenhouse to particular biologic forms of stem rust as a means of isolating  $F_3$  families which have the resistance of the Kota parent under field conditions.

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<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 1012.

## THE PRESENT STATUS OF THE PROBLEM

For reviews of earlier studies which have led to the present mode of attack, the reader is referred to previous papers (3, 4). In the present paper it seems sufficient to sum up the facts now at hand without making an extended review of literature.

Thirty-seven biologic forms of stem rust have been discovered (6), and of these 21 have been found in the upper Mississippi Valley. If the resistance of a wheat variety to each of these biologic forms was due to a separate set of independently inherited factors the problem would be a very difficult one. If the resistance to one biologic form was due to a genetic factor which was allelomorphic to a factor for resistance to a second form the problem of obtaining a wheat resistant to all biologic forms would be an impossibility. No variety of common wheat has been found which is resistant to all biologic forms of rust; but Khapli, an early maturing emmer, is resistant to all 37 forms.

Crosses of Khapli with common varieties have given self-sterile  $F_1$  progeny. This has led to the attempt to transfer the resistance of Khapli to a durum variety, with the hope of then transferring this resistance to common wheats by a subsequent cross.

That the resistance of durum wheats can be transferred to common wheats has been shown. Several years ago a cross was made between Iumillo and Marquis. There was found to be a strong linkage between the durum characters and resistance although this was overcome by growing large numbers. Several plants were obtained from an  $F_2$  population of from 20,000 to 30,000 plants which appeared to have the spike characters of common wheat and which also appeared rust resistant. Selection has been practiced further and the wheats thus obtained appear of desirable field type and have shown high resistance in field experiments.

Kanred, a rust-resistant winter wheat, produced at the Kansas station, proved immune from 11 of the 21 biologic forms of rust which are found in the North-Central States. This immunity was transferred to spring wheats by crosses between Kanred and Marquis. The immunity from all 11 biologic forms was apparently due to a single genetic factor (1, 2).

Puttick (5), in a study of the  $F_2$  progeny of a cross between Mindum and Marquis, obtained seedling wheats which combined the resistance of both parents. In this study, two biologic forms of rust were used to which Marquis and Mindum reacted reciprocally, one parent being resistant and the other susceptible to the respective rust forms. Puttick, however, did not grow the seedlings to maturity so it was impossible to say whether they were of common or durum types.

In the paper preceding this, Harrington and Aamodt (2a) have presented the results of a cross between Pentad, which is resistant to biologic Form XXXIV and susceptible to biologic Form I, and Mindum, which reacts in a reciprocal way to these rust forms. The resistance of Pentad to Form XXXIV and of Mindum to Form I appears to be due to a single main genetic factor in each case. These two factors appear to be independently inherited and in the production, from the cross Pentad  $\times$  Mindum, of a wheat which is resistant to both biologic forms, it is only necessary to grow enough plants to obtain the necessary recombination of the two factors for resistance.

The brief outline given shows the present status of the problem, the mode of attack being to build up synthetically, by certain crosses, wheats which are resistant to as many biologic forms as possible. For this mode of attack a knowledge of the number and nature of the inherited factors is essential.

#### MATERIAL AND METHODS

Certain general methods of work have been used as in previous studies. For this reason only brief descriptions will be made.

Pure lines of the parental varieties were used in making the crosses. The  $F_1$  generation plants were grown in the greenhouses<sup>3</sup> at Washington, D. C., during the winter of 1920-21 and the  $F_2$  generation was grown during the summer of 1921. The season was a very unfavorable one and the seed produced was rather badly shrivelled in certain cases. Individual plant data on spike characters, average seed length, seed plumpness, and seed texture were taken and the  $F_3$  seedlings obtained from sowing seed of individual  $F_2$  plants were inoculated in the greenhouse with the rust forms chosen.

In deciding which rust forms<sup>4</sup> to use, a study was made of the degrees of infection of seedlings of Marquis and Kota when inoculated at the same time and handled in a similar manner.

Eleven biologic forms were available in the greenhouse and all were used in inoculations for the purpose of discovering two to which the two wheat varieties reacted reciprocally. The reactions obtained on Marquis and Kota are given in Table I. Although Kota is rather highly resistant as tested under field conditions, the difference in seedling infection of Marquis and Kota in the greenhouse is not very great. In nearly all cases, however, where there is a difference, Kota shows a lower degree of infection than Marquis. The meaning of the symbols used to designate the types or degrees of infection is given after Table I.

TABLE I.—Reaction of Kota and Marquis to 11 forms of stem rust, expressed by type of infection produced

variety.	Type of infection produced by biologic form of rust No.—										
	I.	III.	IX.	XVII.	XVIII.	XIX.	XXI.	XXVII.	XXIX.	XXXII.	XXXIV.
Marquis.....	4	4-	3	4	4	1 and 2	4	2+	4	3+	3+
Kota.....	3+	3+	3	4-	3	3	3+	0	3+	3	3

<sup>3</sup> The writers are indebted to Mr. J. Allen Clark, of the Office of Cereal Investigations, for growing the  $F_1$  generation and returning seed of the same.

<sup>4</sup> The biologic forms used were obtained from M. N. Levine, who kindly made them available for our use

*Explanation of classes of host reaction and of corresponding types of infection resulting from inoculation of seedling wheat plants with spores of Puccinia graminis tritici*<sup>5</sup>

Classes of host reaction.	Types of infection.
Immune (immune in the field).....	0. No uredinia developed but definite hypersensitive areas present.
Very resistant (highly resistant under field conditions).....	1. Uredinia minute and isolated; surrounded by sharply-defined, continuous, hypersensitive areas; hypersensitive areas lacking uredinia also may be present.
Moderately resistant (highly resistant under field conditions).....	2. Uredinia isolated and small to midsized; hypersensitiveness present in the form of necrotic areas in circles; uredinia often surrounded by green islands.
Moderately susceptible (somewhat resistant in the field).....	3. Uredinia midsized; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.
Very susceptible (entirely susceptible under field conditions).....	4. Uredinia large or varying from midsized to large, numerous and confluent; true hypersensitiveness entirely absent, chlorosis seldom present.

The studies which have been made show that a pure-line wheat variety inoculated with a single biologic form frequently shows a variation in infection from type 3 to type 4 and occasionally from type 2 to type 3, due to varying environmental conditions to which the seedlings are exposed, even when the conditions of the greenhouse test are kept as uniform as possible. These variations apparently are not due to changes in the genotype.

The most striking differences in the infection of Kota and Marquis were obtained with Form XIX and Form XXVII and accordingly these two forms were chosen for the study. Form XIX was collected in Pocatello, Idaho, in November, 1918, and has been cultured through approximately 51 urediniospore generations. The Form XIX used to inoculate the  $F_4$  material was collected in North Dakota in July, 1921, and has been cultured through approximately 17 generations. Form XXVII was collected in Barges, France, in August, 1919, and has been cultured through approximately 45 urediniospore generations.

Between 15 and 20 seeds of each individual  $F_2$  plant were sown in a single pot, but, owing to the unfavorable conditions under which the  $F_2$  plants were grown, the germination of these seeds was low. No pots were used in the study in which the number of  $F_3$  plants per pot fell below 8. While 8 plants is much too small a number to prove absolutely the genetic nature of the individual  $F_2$  parent, it gives a fair indication of the genetic class in question. Larger numbers of seeds could not have been used without depleting the quantity of seed reserved for sowing to obtain  $F_3$  families under field conditions. The results obtained with the small numbers of seed used are reliable enough, however, to furnish a good idea of the approximate number of factors involved, and, from the plant-breeding standpoint, the information obtained is sufficient to answer the main questions for which the investigation was undertaken. It is recognized that from the genetic standpoint larger numbers of seedlings in each  $F_3$  family should be inoculated.

<sup>5</sup> These descriptions of types of infection in the greenhouse are based on those given by Stakman and Levine (6).

One of the problems which it was hoped to solve was the discovery of a greenhouse test of  $F_3$  seedlings which would give an accurate estimate of the behavior of Marquis  $\times$  Kota  $F_3$  families under field conditions. As no such test was available, the larger part of the Marquis  $\times$  Kota material was grown in the rust nursery and an artificial epidemic of stem rust induced by the use of the available biologic forms of stem rust which were present in the greenhouse and which were known to have been found in the spring-wheat region. The forms of rust used for the field epidemic in 1922 are those listed in Table 1, with the exception of Form XXVII, which was originally collected in Barges, France, and Form XXXIV which was collected in South Africa. These two forms were not used for the nursery epidemic.

Between 25 and 50 plants were grown in the field nursery from each selected  $F_3$  family. The rust infection was heavy on susceptible types, as shown by the infection on Marquis, and consequently the determination of those  $F_3$  families which had the Kota type of resistance to the nine biologic forms in question could be made with accuracy.

## EXPERIMENTAL RESULTS

### DWARF PLANTS OBTAINED IN THE $F_2$ PROGENY

The  $F_2$  material of the Marquis  $\times$  Kota cross grown in the plant-breeding nursery in 1921 was not severely infected with stem rust and, therefore, was used for the greenhouse studies. Considerable material also was grown in the rust nursery. With most hybrid material in which Kota had been used as one parent, dwarf plants appeared in the  $F_2$  generation. The frequency of the appearance of such plants was determined for the  $F_2$  progeny which grew in the rust nursery in 1921. Of a total of 787  $F_2$  plants, 666 were of normal type and 121 were dwarfs. Seed from some of these dwarfs was sown in the greenhouse and only dwarf plants resulted. The results may be explained genetically by the assumption that one of the parents contains two factors, one for dwarf habit and the other which inhibits the expression of the dwarf factor. On this basis a 13:3 ratio of normals and dwarfs is expected in  $F_2$ . The number of dwarfs expected in a population of 787 would be 147. The number of dwarf plants obtained was somewhat less than the expectation for a 13:3 ratio.

### THE AWNLESS OR BEARDED CHARACTER IN RELATION TO SEED CHARACTERS

Kota is a bearded wheat, while Marquis has short apical awns and is commonly classified as awnless. The  $F_1$  hybrids have somewhat longer apical awns than Marquis. At harvest time some of these tip awns were broken off and it was impossible to separate the awnless from the hybrid types with certainty. For this reason awnless and apically-awned (hybrid) forms were placed together. In an earlier study of a cross between Marquis and Preston, experimental data gave reason for the belief that the awn was an important physiological organ under the conditions of the experiment. Accordingly a somewhat similar study was made of the Kota  $\times$  Marquis plants (Tables II, III, IV).



TABLE II.—Distribution of  $F_2$  plants of the cross between Marquis and Kota into classes according to average length of seed, in relation to plant and spike characters

Plant type.	Classes for seed length (in millimeters).						Total plants.	Mean.
	4.8	5.1	5.4	5.7	6.0	6.3		
Normal:								
Awnless.....		12	72	174	114	12	384	$5.73 \pm 0.01$
Bearded.....		2	11	51	64	9	137	$5.85 \pm 0.01$
Dwarf:								
Awnless.....	5	9	5	3	1	1	24	$5.26 \pm 0.07$
Bearded.....	3	3	6	.....	.....	.....	12	$5.18 \pm 0.05$

TABLE III.—Distribution of  $F_2$  plants of the cross between Marquis and Kota into classes according to the percentage of hardness of seed, in relation to plant and spike characters

Plant type.	Classes for percentage of hardness (100=corneous).								Total plants.	Mean.
	40	45	50	55	60	65	70	75		
Normal:										
Awnless.....	2	.....	53	119	162	24	23	1	384	$58.92 \pm 0.18$
Bearded.....	.....	1	24	51	46	10	5	.....	137	$57.01 \pm 0.29$
Dwarf:										
Awnless.....	.....	.....	13	11	7	2	.....	.....	33	$54.70 \pm 0.54$
Bearded.....	.....	.....	3	8	3	.....	.....	.....	14	$55.00 \pm 0.04$

TABLE IV.—Distribution of  $F_2$  plants of the cross between Marquis and Kota into classes according to the percentage of plumpness of seed, in relation to plant and spike characters

Plant type.	Classes for percentage of plumpness.													Total plants.	Mean.
	30	40	45	50	55	60	65	70	75	80	85	90			
Normal:															
Awnless.....	2	2	...	13	...	30	14	82	115	36	86	4	384	74.02 ± 0.33	
Bearded.....	...	...	...	1	...	9	6	31	33	33	18	6	137	75.44 ± 0.44	
Dwarf:															
Awnless.....	...	5	1	11	1	4	2	4	5	...	...	...	33	56.82 ± 1.38	
Bearded.....	...	...	1	3	1	1	2	5	1	...	...	...	14	61.79 ± 1.73	

Apparently the presence of the awn results in the production of a somewhat longer seed on the normal plants, the average difference being about 0.1 mm. The texture of the seeds of the awnless normal plants was somewhat harder than that of those on the bearded plants. The percentage of plumpness of the seed of the bearded normal plants averaged slightly higher than in the case of the awnless plants although, in the light of the probable error, the difference obtained is not significant. In the determinations of texture, 100 is the standard for completely corneous seed, the border line between subcorneous and soft being around 50. The percentage of plumpness of seed is taken in a somewhat similar manner.

REACTION OF  $F_2$  SEEDLINGS AND THEIR PARENT VARIETIES TO BIOLOGIC FORMS XIX AND XXVII

Pots of the parent varieties, Marquis and Kota, inoculated and handled in the same manner as the hybrid  $F_2$  families, were used as controls. In order to assist in the study the parents and the various  $F_2$  families were classified into groups on the basis of their resistance or susceptibility to infection by these two forms of rust as follows:

*Classes of resistance and susceptibility to infection in the Marquis-Kota cross when  $F_2$  seedling families and the parent varieties were inoculated with biologic Forms XIX and XXVII in the greenhouse in the winter of 1921-22*

## BIOLOGIC FORM XIX

IR=Resistant in classes of infection types 1 to 2, or that of the Marquis parent.  
 HR=Heterozygous with the Marquis or types 1 and 2 reaction dominant.  
 H=Heterozygous with the Kota or type 3 reaction dominant.  
 HS=Reaction mainly of the Kota type, type 3, with an occasional plant of the Marquis type of reaction.  
 IS=Reaction of type 3, like the Kota parent.

## BIOLOGIC FORM XXVII

R=Infection of type 0, or the immunity of the Kota parent.  
 IR=Reaction of type 2, the resistance of the Marquis parent. The uredinia were somewhat larger than for the normal type 2 infection.  
 H<sub>1</sub>=Heterozygous with reactions of types 0 and 2.  
 H<sub>2</sub>=Heterozygous with reactions of types 0, 2, and 4.  
 H<sub>3</sub>=Heterozygous with reactions of types 2 and 4.  
 H<sub>4</sub>=Heterozygous with reactions of types 0 and 4.  
 S=Susceptible with reaction in type 4 only.

The results obtained from inoculating the parents and  $F_2$  hybrids with biologic Form XIX are given in Table V.

TABLE V.—Distribution of  $F_2$  seedlings of the cross between Kota and Marquis according to types of infection caused by biologic Form XIX

Classes of parents and $F_2$ crosses.	Distribution by types of infection.										Number of plants.	Number of families.
	0	-1	1	1+	-2	2	2+	-3	3	3+		
$F_2$ families IR....	4	17	170	18	14	150	39	.....	.....	.....	412	40
$F_2$ families HR....	9	1	147	15	13	163	15	13	101	1	478	44
$F_2$ families H....	23	.....	118	8	15	187	27	123	768	.....	1,269	114
$F_2$ families HS....	7	1	20	3	.....	17	3	45	381	.....	477	45
$F_2$ families IS....	35	.....	.....	.....	.....	.....	.....	131	2,117	25	2,308	212
Marquis IR.....	5	.....	215	1	.....	40	3	.....	.....	.....	264	23
Marquis HR.....	16	.....	326	39	12	107	9	33	83	2	627	50
Kota HS.....	.....	.....	1	.....	.....	.....	.....	.....	15	.....	16	1
Kota IS.....	13	.....	.....	.....	.....	.....	.....	3	962	.....	978	81

The infection of the Kota parent fell within type 3 with the exception of one plant which had a type 1 infection. With the conditions under which the studies were made, occasional infection with a different biologic form could not be avoided. The infection of the Marquis parent fell mainly within types 1 and 2, although there were 118 plants out of 891 which showed infection of type 3.

The Marquis seed used was from a carefully selected sample and was typical of the Marquis variety. There was the possibility that these Marquis seedlings which in the greenhouse gave type 3 infections were of a different genetic nature than the greater part of the Marquis seedlings, due to mixtures or some other causes. Accordingly, seedlings which showed the type 3 and the type 1 or 2 infection in the greenhouse were planted in the field and grown to maturity. All plants appeared to be of the Marquis variety. Seeds from several plants in each group were saved separately and progeny of these plants tested in the greenhouse by inoculating with spores of Form XIX. Five out of six of the progeny of the seedling plants which had type 3 infections again gave seedlings which were placed in the 3 group, while the infection of one family was mainly in types 1 and 2. Six out of seven progeny lines which descended from seedlings having type 2 infections again had similar degrees of infection, while one family bore infection mainly of type 3. The reason, then, for the greater part of the type 3 infections obtained in the Marquis seedlings appears to be genetic differences in the Marquis material.

To determine their reaction to biologic Form XIX, 455  $F_3$  families were tested. Some proved homozygous in the moderately resistant and the moderately susceptible types. Other  $F_3$  families proved to be heterozygous. The number of susceptible types is too large to be explained on the basis of a single factor difference. Forty resistant families were obtained.

Certain of the  $F_2$  parent plants of these various  $F_3$  families were selected and their progeny grown in the rust nursery in 1922. Some lines appeared desirable and individual plants within these lines were selected. Seedlings were grown in the greenhouse from the individually selected plants and were inoculated with Form XIX. Progenies of 3, 4, and 5 plants of three different  $F_3$  families, which were classified as IS, or of the Kota type of infection, were grown in the greenhouse. All 12 plants bred true to the IS type of infection. One family from the IR group was tested in a similar manner. Progeny of 5 plants of this family were tested, and all showed type 2 infections, as was expected.

The reactions to Form XXVII were of a more definite nature than those to Form XIX, as will be seen in Table VI. Of the Kota plants all except 33 out of a total of 1,040 were in the immune class, while Marquis had 9 plants in type 4 and 725 in type 2 infections. The deviations obtained could all be explained on the basis of natural causes, for the frequency of such natural crossing is in the neighborhood of 2 to 4 per cent in Minnesota under nursery conditions.

A total of 462  $F_3$  families were tested. Of these, 76 showed either no infection or infection of the 0 or immune type, 14 were resistant, and 39 were susceptible, and there were four separate classes of heterozygous types. Immunity is clearly dominant over resistance or susceptibility as shown by the results for the heterozygous classes  $H_1$  and  $H_4$ .

The segregating classes obtained when  $F_3$  families were inoculated with Form XXVII could result from two independently inherited genetic factors for resistance and immunity each allelomorphic to a factor for susceptibility. Furthermore, if the factor for immunity was epistatic to the factor for resistance, three times as many homozygous immune families would be obtained as homozygous for either resistance or susceptibility. It is possible that a much more complex genetic condition may be the cause of the classes of segregation obtained. The clear proof that

some susceptible types may be obtained from a cross of resistant parents is of much interest.

TABLE VI.—*Distribution of the  $F_3$  seedlings of the cross between Kota and Marquis according to types of infection caused by biologic Form XXVII*

Classes of parents and $F_3$ crosses.	Types of infection.						Number of plants.	Number of families.
	0	2-	2	2+	4-	4		
$F_3$ families R. ....	1,159						1,159	76
$F_3$ families IR. ....	12		55	88			155	14
$F_3$ families H <sub>1</sub> . ....	588	2	66	113			769	62
$F_3$ families H <sub>2</sub> . ....	707		52	113	164	29	1,065	82
$F_3$ families H <sub>3</sub> . ....	23	23	67	225	264	20	631	50
$F_3$ families H <sub>4</sub> . ....	1,312				305	98	1,715	139
$F_3$ families S. ....	22				387	87	496	39
Marquis H <sub>3</sub> . ....			28	67	8	1	104	8
Marquis IR. ....	9		172	458			639	55
Kota H <sub>1</sub> . ....	16		1				17	1
Kota H <sub>4</sub> . ....	179				32	1	212	15
Kota R. ....	811						811	59

Several hybrid  $F_3$  families, whose seedling reaction to Form XXVII was known, were grown in the rust nursery in 1922, and individual plants from various progeny lines were selected. Three such lines, which in a previous greenhouse test gave reactions which placed them in the R or immune group, were again tested for their reaction to Form XXVII for the purpose of checking up the accuracy of the preceding year's study. A total of 16 plants belonging to these three families had type 0 infection and thus were in the immune class. Two lines of the susceptible group were similarly tested. One gave evidence of breeding true for susceptibility while another segregated, giving both susceptible and resistant types. If similar results were obtained, the number of families in the susceptible group should be decreased from that presented in Table VI.

The Marquis and Kota pots which were inoculated at the same time were used as controls on the purity of the biologic forms. In the hand-inoculated pots, the killed areas around the uredinia on Marquis were much less sharply defined than in the studies made the previous winter. In two pots, Marquis seedlings which were inoculated by brushing with seedlings infected with biologic Form XXVII were heavily infected with rust over the greater part of their leaf surface. The uredinia on these heavily infected seedlings were surrounded by sharply defined hypersensitive areas. Apparently environmental conditions may influence, to a rather marked degree, the reaction of seedlings to a particular biologic form.

#### INFECTION UNDER FIELD CONDITIONS

One of the purposes of the investigation was to determine the possibility of using the greenhouse test as a means of determining those  $F_3$  families having the type of resistance which Kota shows under field conditions. While Kota in the field frequently is heavily infected with stem rust, the uredinia do not break out in the same manner as on susceptible varieties and are much smaller and often surrounded by a

hypersensitive area. For this reason the seed of Kota is generally well filled even under rust-epidemic conditions.

The  $F_3$  families studied in their reaction to Form XIX and Form XXVII in the greenhouse were the progeny of  $F_2$  plants taken at random from an  $F_2$  population. The seed characters of these plants were examined and the plants which appeared to have desirable seed from an agronomic standpoint were selected and their progeny grown in the rust nursery in 1922. The various  $F_3$  lines were exposed, as has been previously noted, to at least 9 biologic forms of stem rust. The epidemic was a satisfactory one. Individual plants within the different  $F_3$  families were carefully examined and on the basis of the infection obtained the  $F_3$  families were placed in the following classes:

- R=Resistant; while there was considerable infection the plants all showed the Kota type of resistance.  
 NR=Semiresistant, all plants infected in a similar way. Much more resistant than Marquis, but more heavily infected than Kota.  
 H=Heterozygous, containing both resistant and susceptible plants.  
 S=Susceptible, all plants heavily infected, with large uredinia, similar to the infection shown by Marquis.

The results of the field experiment are presented in Table VII. The 9 control plots of Kota were all clearly resistant while the 20 control plots of Marquis were fully susceptible.

TABLE VII.—Distribution of 206  $F_3$  families of the Marquis-Kota cross, which had not been tested previously under rust-epidemic conditions, when inoculated in the rust nursery with a mixture of 9 different rust forms, in 1922

Parents or hybrids.	Number of families.			
	Homozygous.		Heterozygous.	Susceptible.
	Resistant.	Semi-resistant.		
$F_3$ awnless homozygous .....	3	18	3	22
$F_3$ bearded homozygous .....	15	35	16	22
$F_3$ heterozygous .....	10	33	12	17
Kota, bearded .....	9	.....	.....	.....
Marquis, awnless .....	.....	.....	.....	20

Of a total of 206  $F_3$  families 28 were homozygous resistant, 61 were susceptible, 86 appeared semiresistant, and 31 were clearly heterozygous. These results can not be explained on a single factor basis, because only 28 out of 206, which is a ratio of 1 to 7.36, were as resistant as Kota. It should be remembered that 9 biologic forms of rust were used to produce the field epidemic. Apparently the Kota type of resistance to all 9 forms depends on more than a single genetic factor.

In 1921 an  $F_2$  generation of the Kota-Marquis cross was planted in the rust nursery. Several of the 9 biologic forms used in 1922 to produce the epidemic were available, although only three or four biologic forms were used to produce the 1921 epidemic. A total of 666 normal plants, which were carefully examined, consisted of 80 bearded susceptible, 233 awnless susceptible, 89 bearded apparently resistant, and 264 awnless apparently resistant. The 353 plants which appeared resistant were harvested and on examination 47 appeared to have desirable seed char-

acters. Progenies of these were grown in 1922 in the field and the rust reaction determined. Eleven of the 47 families were resistant, 14 were semiresistant, 4 were susceptible, and 12 were heterozygous. If the same relative proportion of the entire 353 resistant families had bred true to resistance there would have been 82 out of 666  $F_2$  plants that were homozygous in their resistance to all eight biologic forms. This is a ratio of 1 homozygous resistant to 8.1 of other types, which is very similar to the previously discussed result of the inoculation of 206  $F_2$  families of which 28 bred true for the Kota type of resistance.

#### THE GREENHOUSE TEST AS A MEANS OF ISOLATING LINES RESISTANT IN THE FIELD

One of the purposes of a study of the greenhouse reactions to Forms XIX and XXVII was to determine the possibility of using this test as a means of isolating lines which would exhibit the Kota type of resistance in the field. Fifty-two families whose reactions to Form XIX were known in the greenhouse and 48 families whose reactions to Form XXVII were known were grown in the field nursery in 1922 and classified under field conditions as resistant, near-resistant, heterozygous, and susceptible, as shown in Table VIII.

TABLE VIII.—*Distribution into classes according to reaction in the greenhouse of  $F_2$  seedlings from  $F_2$  plants in relation to behavior of  $F_2$  families in the field rust nursery under artificial epidemic conditions*

Reaction under field conditions.	Greenhouse reaction—											
	To Form XIX.					To Form XXVII.						
	IR.	HR.	H.	HS.	IS.	R.	IR.	H <sub>1</sub> .	H <sub>2</sub> .	H <sub>3</sub> .	H <sub>4</sub> .	S.
R.....	0	1	1	0	3	1	1	0	0	0	3	0
NR.....	1	3	10	2	11	5	0	2	6	3	5	3
H.....	3	0	2	1	4	2	2	0	1	0	3	1
S.....	1	0	4	1	4	0	0	4	2	0	2	0

A study of the results shows that the greenhouse reactions to the biologic Forms XIX or XXVII can not be used to determine which families will exhibit the Kota type of resistance to several biologic forms in the field. Thus of the five families which gave the Marquis type of reaction to Form XIX in the greenhouse, namely, the IR group, none bred true for resistance under field conditions. Similarly, of the 22 families which gave the Kota type of reaction in the greenhouse and which were placed in the IS group, only 3 proved resistant in the field. The reaction of Kota to Form XXVII was of the immune or R type. Eight hybrid families of this type were grown in the field and only one proved as resistant as Kota. These results are further evidence which proves that the resistance of Kota to several biologic forms of stem rust is due to more than a single genetic factor.

QUESTION AS TO WHETHER THE RESISTANCE TO FORM XIX AND FORM XXVII  
CAN BE COMBINED IN A SINGLE VARIETY

In order to build up wheats resistant to all biologic forms, it is necessary to combine in a single variety the resistance exhibited by different wheat varieties to particular biologic forms. The reactions of  $F_2$  families to Forms XIX and XXVII are correlated in Table IX.

TABLE IX.—*Distribution of  $F_2$  families classified according to reaction to form XIX in relation to reaction to Form XXVII*

Reaction to Form XXVII.	Reaction to Form XIX.					
	IR.	HR.	H.	HS.	IS.	Total.
R.....	3	9	13	5	44	74
IR.....	4	2	1	0	1	8
H <sub>1</sub> .....	6	6	12	4	14	42
H <sub>2</sub> .....	7	11	13	7	29	67
H <sub>3</sub> .....	1	5	15	3	13	37
H <sub>4</sub> .....	3	9	27	15	59	113
S.....	1	2	8	1	19	31
Total.....	25	44	89	35	179	372

Three out of a total of 372 families proved immune from Form XXVII and were resistant to Form XIX, and 4 families were resistant to both biologic Forms XIX and XXVII. It is possible, therefore, to combine in a single variety the resistance of the Marquis parent to Form XIX, and the immunity of the Kota parent from Form XXVII. These facts give further hope for the belief that resistance to different biologic forms exhibited by different wheat varieties is due to independently inherited Mendelian factors or that the linkage of these factors is so slight that crossovers frequently occur. From the genetic standpoint the facts so far learned give reason for the belief that a wheat variety can be produced which will be resistant to all biologic forms of stem rust.

CORRELATION BETWEEN MORPHOLOGIC AND SEED CHARACTERS AND RUST  
RESISTANCE

The  $F_2$  plants, which were the parents of the  $F_3$  families tested under field conditions were classified as normal awnless or bearded and dwarf awnless or dwarf bearded. For the purpose of determining whether there was any marked correlation between these characters and reaction to rust, the reactions of these separate morphologic groups to Form XIX and Form XXVII have been arranged in correlation, as shown in Tables X and XI.

TABLE X.—Distribution of  $F_2$  normal awnless (A) and bearded (B) plants and dwarf awnless (DA) and bearded (DB) plants, classified according to their reaction to Form XIX in relation to spike characteristics of  $F_2$  plants

F <sub>2</sub> plant type.	F <sub>2</sub> reaction to Form XIX.				
	IR.	HR.	H.	HS.	IS.
A.....	28	35	78	28	142
B.....	10	12	23	11	46
DA.....	1	0	12	1	19
DB.....	0	2	3	1	5
Total.....	39	49	116	41	212

TABLE XI.—Distribution of  $F_2$  normal awnless (A) and bearded (B) plants and dwarf awnless (DA) and bearded (DB) plants, classified according to their reaction to Form XXVII in relation to spike characteristics of  $F_2$  plants

F <sub>2</sub> plant type.	F <sub>2</sub> reaction to Form XXVII.						
	R.	IR.	H <sub>1</sub> .	H <sub>2</sub> .	H <sub>3</sub> .	H <sub>4</sub> .	S.
A.....	62	11	47	60	32	101	27
B.....	28	3	11	20	11	30	8
DA.....	2	0	1	1	2	5	2
DB.....	1	0	1	2	2	2	2
Total.....	93	14	60	83	47	138	39

Three times as many awnless families as bearded were expected. The fact that there were about three times as many awnless as bearded families in each of the groups classified according to reaction to both biologic forms seems very convincing proof that the factors which govern susceptibility or resistance to either Form XIX or Form XXVII are inherited separately from the factors which govern the presence or absence of awn development.

To Form XXVII the reaction of the  $F_2$  families from the dwarf types seems very similar to that obtained from normal plants. For Form XIX, however, the percentage of dwarf plants which were as resistant as Marquis and which were placed in the IR group is very small and certainly much less than the percentage of similar families from the normal plants.

In a somewhat similar manner the relation between average length of seed of the  $F_2$  plants and the reaction of these plants to Forms XIX and XXVII was determined, as shown in Tables XII and XIII.



TABLE XII.—Distribution of  $F_2$  plants classified according to their reaction to Form XIX in relation to length of seed

Classes by reaction to Form XIX.	Classes for length of seed (in millimeters).						Total.	Mean.
	4.8	5.1	5.4	5.7	6.0	6.3		
IR.....			5	17	9	3	34	5.79±0.03
HR.....	1	3	5	21	18	1	49	5.74±0.03
H.....	3	7	18	50	30	2	110	5.69±0.02
HS.....	1		6	18	15		40	5.75±0.03
IS.....	2	10	33	81	67	9	202	5.74±0.01

TABLE XIII.—Distribution of  $F_2$  plants classified according to their reaction to Form XXVII in relation to length of seed

Classes by reaction to Form XXVII.	Classes for length of seed (in millimeters).						Total.	Mean.
	4.8	5.1	5.4	5.7	6.0	6.3		
R.....		2	15	46	24	6	93	5.75±0.02
IR.....			4	5	3	2	14	5.77±0.06
H <sub>1</sub> .....	2	3	8	25	20	2	60	5.72±0.03
H <sub>2</sub> .....		2	17	32	28	3	82	5.75±0.02
H <sub>3</sub> .....	1	4	11	19	10	1	46	5.63±0.03
H <sub>4</sub> .....	1	3	17	60	52	2	135	5.77±0.01
S.....		4	5	15	13	1	38	5.72±0.03

The ranges of variability for seed length are somewhat greater, as would be expected, for the reaction groups which contain the largest number of families. The differences obtained in the means are not sufficiently great to indicate any marked linkage between the factors of inheritance which govern seed length and those which govern the manner of reaction to the forms of stem rust used in the study.

#### SUMMARY

(1) Kota, which is resistant to stem rust in the field but which has weak straw when grown under humid conditions, was crossed with Marquis, which is susceptible to stem rust but which possesses other desirable economic characters.

(2) A survey of available biologic forms led to the selection of Form XIX, to which Marquis was resistant and Kota moderately susceptible, and Form XXVII, from which Kota was immune and to which Marquis was resistant. These two forms of stem rust were chosen for the study with the hope of finding a greenhouse test which could be used as a means of isolating  $F_2$  families which would prove as resistant as Kota under field conditions.

(3) Out of a total of 787  $F_2$  plants of the Kota-Marquis cross which were grown under field conditions, 666 were of normal type and 121 were dwarfs. The results were explained on the basis that one of the parents contained a factor for dwarf habit and another factor which prevented the production of dwarfs. The other parent apparently lacked both factors.

(4) The seeds of the  $F_2$  bearded plants were 0.1 mm. longer on an average than the seeds from the awnless plants. The seeds of the awnless plants had slightly harder texture but were slightly inferior in plumpness to those from the bearded plants. Apparently the beard of wheat, under the conditions of the experiments, leads to the production of somewhat better developed seed.

(5) Marquis, in the greenhouse, was more resistant than Kota to Form XIX. Varying environmental conditions, however, resulted in the production of a type of infection on Marquis which approached that obtained normally with Kota. The reactions of Kota to Form XIX under the same conditions did not show similar variations in the types of infection.  $F_3$  hybrid families were obtained which reacted in a manner similar to Marquis and Kota, while other families were obtained which were clearly heterozygous. The results could not be explained on the basis of a single genetic factor.

(6) Kota proved immune from Form XXVII in the greenhouse, while Marquis proved resistant.  $F_3$  families were obtained which were as homozygous for immunity and resistance as Kota and Marquis, respectively, while others were obtained which were entirely homozygous for susceptibility. Four types of heterozygous families were obtained. Immunity appeared to be dominant to both resistance and susceptibility. The number of seedlings inoculated in each family available for the study was too small to make the experiment absolutely conclusive. The results could be quite satisfactorily explained on the basis of two independently inherited factors for immunity and resistance contained in the Kota and Marquis parents, respectively, each factor being allelomorphous and dominant to a factor for susceptibility.

(7) Nine biologic forms of stem rust which were collected in the North-Central and Northwestern States were used for producing a field epidemic. Kota proved rather highly resistant under field conditions while Marquis was very susceptible. In one group of 206  $F_3$  families there were 28 which were as resistant as Kota. This is a ratio of 1 to 7.36. In another test there was a ratio of 1 resistant  $F_3$  family to 8.1 susceptible and heterozygous.

(8) The reaction of these  $F_3$  families to Forms XIX and XXVII in the greenhouse was correlated with the behavior of such  $F_3$  families under field conditions when an epidemic of stem rust was induced by infection with nine biologic forms of stem rust to which Kota proved resistant in the field. The study of  $F_3$  seedlings in the greenhouse in their reaction to Forms XIX and XXVII was not satisfactory as a means of isolating  $F_3$  lines which would exhibit the Kota type of resistance under field conditions.

(9) The resistance of Marquis to Form XIX and the immunity of Kota from Form XXVII were combined in 3  $F_3$  families out of a total of 372 families studied. This is further evidence in support of the belief that there are several genetic factors which determine the differential reactions of Marquis and Kota to the biologic forms in question. That the resistance of one parent to Form XIX and the immunity of the other parent from Form XXVII can be combined in a single hybrid family is added reason for the hope that resistance to all biologic forms can be obtained eventually.

(10) There was no close linkage in inheritance of the factors which govern the presence or absence of the awns or of seed length with those which determine the resistance or susceptibility to either biologic form used in the greenhouse study.

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PLATE 1

Comparative lodging of Kota and Marquis. At left, Kota, badly lodged; at right, Marquis, fully erect. Photograph taken at University Farm, St. Paul, Minn., 1922.



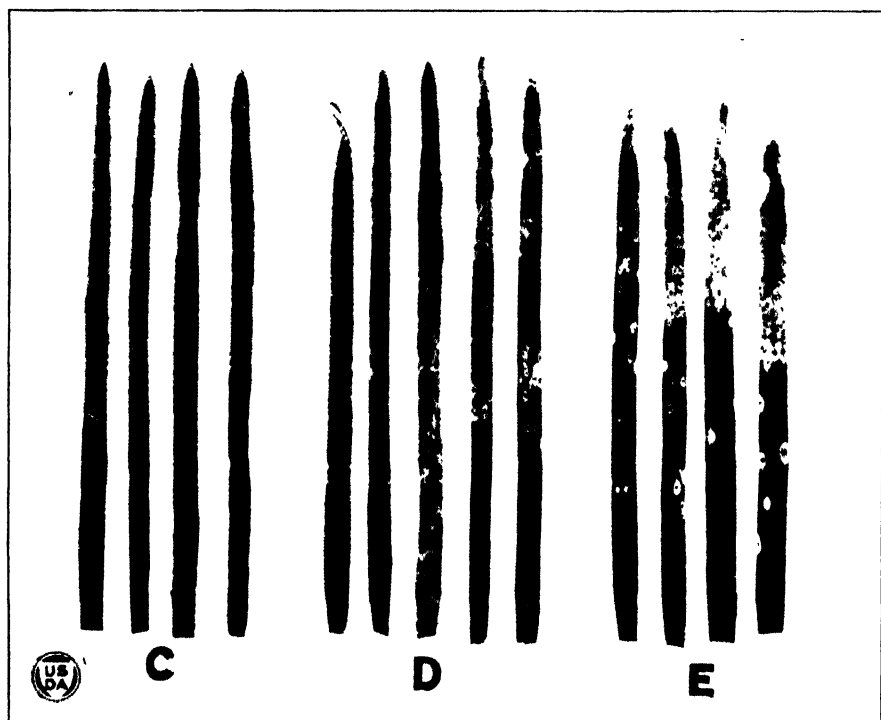
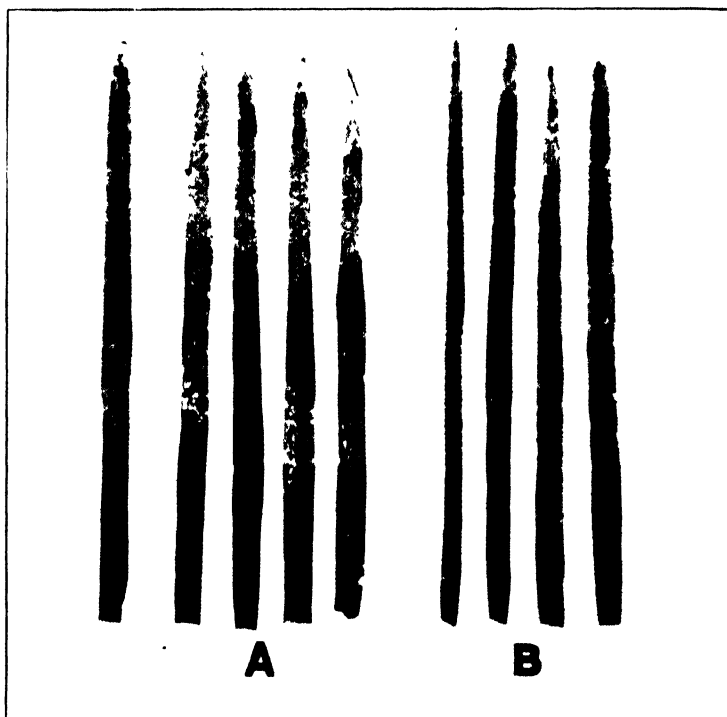


PLATE 2

Reaction of Marquis and Kota and of  $F_3$  families of the Marquis  $\times$  Kota cross to biologic Form XIX.

A.—Reaction of Marquis of types 1 and 2 except the leaf at the extreme left, which proved to have a mixture of infection types.

B.—Reaction of Kota of type 3.

C.— $F_3$  family of the immune-susceptible group.

D.— $F_3$  heterozygous family.

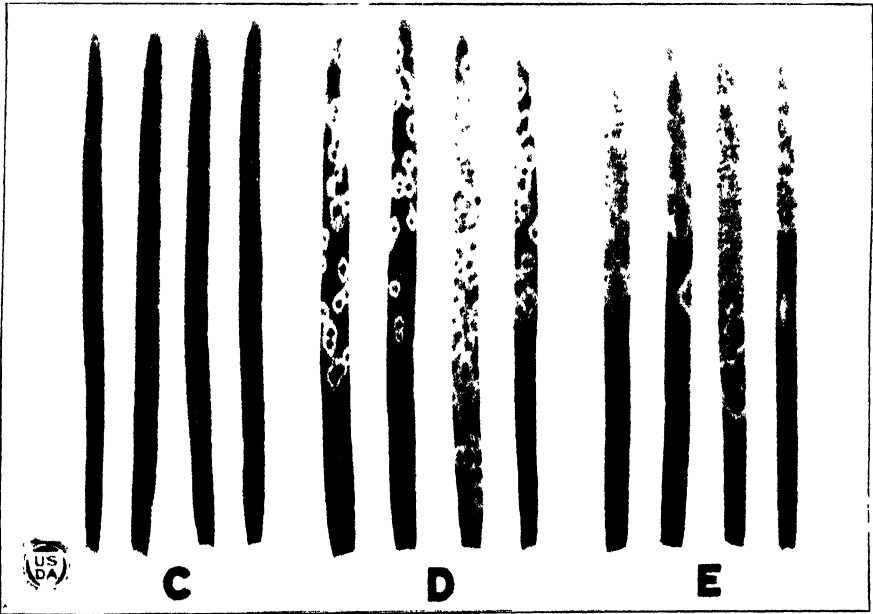
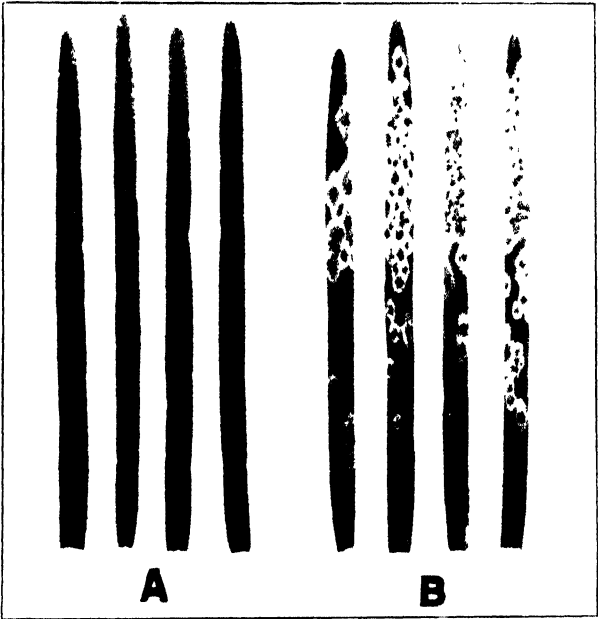
E.— $F_3$  family of the immune-resistant group.



PLATE 3

Reaction of Marquis and Kota and of  $F_2$  families of the Marquis  $\times$  Kota cross to biologic Form XXVII.

- A.—Kota, showing immunity represented by infection of type o.
- B.—Marquis, showing resistance which classifies it in the immune-resistant group.
- C.—Immune family.
- D.—Resistant family.
- E.—Susceptible family.





## BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON VARIETIES OF AVENA SPP.<sup>1</sup>

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*Puccinia graminis tritici* Erikss. and Henn., which originally was considered as a single biologic form, has been shown by Stakman and Piemeisel (11),<sup>3</sup> Levine and Stakman (5), Melchers and Parker (7), Stakman, Levine, and Leach (10), and Stakman and Levine (9) actually to consist of many forms which differ in their parasitic action on certain varieties of *Triticum* spp. Hoerner (4) showed that there is a similar specialization of *Puccinia coronata* Cda. on varieties of *Avena* spp. and Mains and Jackson (6) found two biologic forms of *P. triticea* Erikss. on varieties of wheat. It seemed quite likely, therefore, that *P. graminis avenae* Erikss. and Henn. might also consist of several biologic forms. Experiments, therefore, were begun in the fall of 1918 to ascertain whether this was true.

It is obvious that one of the most direct methods for ascertaining whether there are biologic forms of rust, with different parasitic capabilities on varieties of cereals, is to find forms of rust capable of infecting varieties ordinarily practically immune from described rust forms, or vice versa. As a result of preliminary studies on *Puccinia graminis avenae* it was found that Victory (both C. I. No. 1145<sup>4</sup> and Minn. 514), Improved Ligowa (Minn. 281), Minota (Minn. 512), and many other varieties of oats were extremely susceptible to the rust strains<sup>5</sup> used, while White Tartar (White Russian) (Minn. 339 and two pure-line selections made by the section of plant pathology at the Iowa Experiment Station and designated as Rust Nursery Row No. 101½ and 102½), was quite resistant. The writers, therefore, began collecting uredinial material of *P. graminis* Pers. on oats from as many different localities as possible, in this and other countries.

Since the fall of 1918, more than 100 collections of *P. graminis* on oats have been obtained from 21 States of the United States, from two Provinces in Canada, and from one State in Mexico. Seventy-six of the collections have been cultured in the greenhouse of the section of plant pathology at University Farm, St. Paul, Minn. Many of these were used for inoculating seedlings of about 70 different varieties and selections

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<sup>2</sup> The writers are indebted for rust material to Prof. W. P. Fraser, pathologist in charge of Cereal Disease Investigations, Dominion Laboratory of Plant Pathology, in cooperation with the University of Saskatchewan, Saskatoon, Sask., Canada; to Prof. Wallace Butler, of San Antonio, Tex.; to Mr. G. F. Puttick, of the Department of Agriculture of the Union of South Africa; and to Mr. Fred Griffie, assistant plant breeder, Department of Agriculture, University of Minnesota. They also are indebted to other Federal and State pathologists for collections of rust.

Dr. H. K. Hayes, plant breeder, Department of Agriculture, University of Minnesota and collaborator of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, made valuable suggestions for which the authors wish to express their appreciation.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 1017-1018.

<sup>4</sup> C. I.—Cereal investigations accession number.

<sup>5</sup> The term "strain" is used only to designate a culture of rust from a single collection.

of oats, belonging to 6 different species of the genus *Avena*, namely: *A. brevis* Roth., *A. nuda* L., *A. sativa* L., *A. orientalis* L., *A. sterilis* L., and *A. strigosa* Schreb.

It was found that definite preliminary results were likely to be obtained by using only three varieties of oats, viz: Victory, White Tartar (White Russian) and Monarch Selection (of Etheridge). All collections were run to Victory and White Tartar (White Russian). Victory was completely susceptible to all of the strains tried, White Tartar was very highly resistant to most of them, while Monarch Selection, which was inoculated only with the collections made in 1921 and 1922, was extremely susceptible to some of these collections and practically immune from others. Attempts then were made to find forms of stem rust which would infect the White Tartar variety heavily. This was achieved with rust material procured from Europe and Africa.

Victory was completely susceptible to, White Tartar very resistant to, and Monarch Selection (of Etheridge) practically immune from, a form collected by Mr. Fred Griffie at St. Paul, Minn. This is designated hereafter as Form I.

A form of rust collected by Prof. Wallace Butler at San Marcos, Tex., infected Victory normally, developed only lightly on White Tartar, but produced large, vigorous uredinia on Monarch Selection. These results were confirmed by repeated inoculations, and clearly indicated the existence of a second biologic form of *Puccinia graminis avenae*, as it was capable of infecting Monarch Selection normally, which the form collected by Mr. Griffie could not infect. This is designated as Form II.

A rust strain sent by Mr. G. F. Puttick from Potchefstroom, Union of South Africa, infected Victory normally, White Tartar moderately, and Monarch Selection normally. This undoubtedly was a third form and is hereafter called Form III.

Finally, Stakman found a form at Upsala, Sweden, which infected White Tartar just as heavily as it did Victory and Monarch Selection. This, then, was a fourth form,<sup>6</sup> designated as Form IV.

Table I summarizes the action of these four forms on the varieties of oats which served as the differential hosts.

It is quite evident from Table I that Form I (Pl. 2, A) infects Victory normally, but develops only lightly on White Tartar (White Russian) and exceedingly lightly on Monarch Selection. Form II (Pl. 2, B) infects Victory and Monarch Selection heavily, but attacks White Tartar only lightly. Form III (Pl. 3, A), like Form II, attacks Victory and Monarch Selection very heavily and in addition attacks White Tartar moderately. Form IV (Pl. 3, B) infects all three varieties very heavily. Repeated inoculations have been made and the same results have been obtained consistently.

Two different forms were isolated from a single collection several times. In some cases the X type of infection described for wheat rust (9, p. 5) also developed on oats. Several strains produced this X type of infection on Monarch Selection, thus indicating the probable existence of a fifth biologic form which infects Victory very heavily, White Tartar only lightly and Monarch Selection heterogeneously (Pl. 4). There are now known, therefore, at least four, and probably five, biologic forms of *Puccinia graminis avenae* which produce different reactions on certain varieties of *Avena* spp.

<sup>6</sup> Proper disposition is being made of the spore material of the South African and Swedish forms, as they have not yet been found in the United States.

TABLE I.—Results of inoculating three differential varieties of *Avena* spp., with four biologic forms of *Puccinia graminis* avenue

Biologic Form No.	Place of collection.	Summary of inoculation results.					
		Victory (C. I. No. 1145).		White Tartar (Minn. 339).		Monarch Selection (of Etheridge).	
		Number of trials <sup>a</sup>	Character of infection.	Number of trials.	Character of infection.	Number of trials.	Character of infection.
I	St. Paul, Minn	19	Normal, heavy infection. Uredinia large, numerous and coalescing.	8	Infection light. Uredinia usually small and scattered. Hypersensitiveness (sharp chlorosis to definite necrosis) ordinarily present	6	Infection exceedingly light. Uredinia, when present, minute, scattered and surrounded by very sharply defined necrotic areas. Distinct hypersensitive flecks also occur quite frequently.
II	San Marcos, Tex.	16	.... do ....	8	.... do....	6	Normal, heavy infection. Uredinia large, coalescent and numerous.
III	Potchefstroom, South Africa	7	....do ....	5	Infection moderate. Uredinia medium in size and only slightly confluent. True hypersensitiveness absent, but light chlorotic areas usually present.	3	Do.
IV	Upsala, Sweden.	2	.. do .	2	Normal, heavy infection. Uredinia large, many, and confluent	2	Do.

<sup>a</sup> From 10 to 15 plants were inoculated in each trial.

These newly discovered biologic forms are quite as distinct as are those of *P. graminis* on wheat varieties (12). The inoculations were made on seedlings in the greenhouse, but, as a result of previous work, it is safe to conclude that the reaction of seedlings to rust forms is a fairly accurate index of the reaction of older plants. It usually is more difficult to obtain infection on older plants than it is on seedlings, but this apparently is due very largely to the fact that the film of water necessary for spore germination and entrance of germ tubes through the stomata, is not easily maintained on old plants. However, when this difficulty is overcome, by atomizing the plants several times a day, infection occurs normally.

The new forms apparently are constant. Repeated inoculations and cross inoculations have been made with most of them and the results have been consistent. There are variations, of course, in the intensity of rust development when the environmental conditions are unfavorable for the development of host or parasite or both. When light intensity is low, and when the temperature is either too high or too low, the rust develops subnormally. If the host plants are weakened by poor growing conditions, by mildew, root rots, or other factors, the rust usually does not develop well. This variation, however, does not indicate any change in the genotypic constitution of the rust forms, but is only the temporary result of environment. In order to draw accurate conclusions regarding the parasitic behavior of biologic forms it is necessary to grow the host plants and the rust fungus within the range of environmental conditions in

which they will develop normally. Optimum conditions are not essential but extremely unfavorable conditions should be avoided.

It is important to determine whether the urediniospores of these new forms are really of the avenae type, as *P. graminis phleipratensis* (Erikss. and Henn.) Stak. and Piem. develops moderately well from artificial inoculation on some varieties of oats and may possibly occur on some in the field. *P. graminis agrostis* Erikss. also can attack some oat varieties weakly. These forms, however, can be distinguished easily from *P. graminis avenae* by the morphological characters of the urediniospores. The urediniospore characters of the new forms clearly are those of *P. graminis avenae* (8). However, it is not yet known whether these individual forms differ appreciably from each other morphologically.

The geographical distribution of these biologic forms of *P. graminis avenae* is not yet known definitely. The sources of 23 collections, made during 1921 and 1922, are given in Table II. Form I has been collected from Saskatchewan to Mexico, in the central part of the continent, and probably is widely distributed.

TABLE II.—Distribution of biologic forms of stem rust of oats collected during 1921 and 1922

Biologic Form.	Place of collection.	Original host.
II	Saskatoon, Sask., Canada.....	Avena sativa L.
a V	Lloydminster, Sask., Canada.....	Do.
a V	Weyburn, Sask., Canada.....	Do.
I, II	Zelma, Sask., Canada.....	Do.
II	Presque Isle, Me.....	Do.
I, II	Litchfield, Minn.....	Do.
I, II	Rosetown, Minn.....	Do.
I	St. Paul, Minn.....	Do.
II	Redfield, S. Dak.....	Do.
II	Huron, S. Dak.....	Do.
a V	Colmar, Iowa.....	Do.
I, II	Lafayette, Ind.....	Poa trivialis L.
I	Huntley, Ill.....	Avena sativa.
II	Chillicothe, Mo.....	Do.
II	Norman, Okla.....	Dactylis glomerata L.
II	Lawton, Okla.....	Avena sativa.
II	San Marcos, Tex.....	Do.
I, II	Boerne, Tex.....	Do.
I, II	San Antonio, Tex.....	Do.
I, II	Robards, Tex.....	Do.
I, II	Saltillo, Mexico.....	Do.
IV	Upsala, Sweden.....	Do.
II, III	Potchefstroom, South Africa.....	Do.

\* If not a mechanical mixture of I and II.

Form II seems to be even more abundantly distributed than Form I in the central part of North America, from Saskatchewan to Mexico, and has been found also in Maine, as well as in South Africa.

Forms III and IV have not yet been found in North America. It seems improbable that they are abundant in the northern Mississippi Valley, because the White Tartar (White Russian) oat is very resistant in the field. Furthermore, Garber (3) produced Victory × White Russian hybrids which were resistant to stem rust, at least in Minnesota. As these

hybrid strains combine the desirable characters of Victory with the rust resistance of White Tartar, it is to be hoped that Forms III and IV of *P. graminis avenae* do not exist in the United States. If they do not, the problem of developing good varieties of oats resistant to stem rust seems to be well on the way to solution.

The provisional Form V was collected in two localities in Saskatchewan, Canada, and in one in Iowa. It may be a mechanical mixture of Forms I and II. Inoculation experiments to determine this fact are in progress.

It is especially interesting to note that the Swedish form of rust is more virulent than any of those so far found in the United States, because Eriksson (2) long ago called attention to the fact that *P. graminis avenae* was exceptionally virulent in Sweden. The senior author was also impressed with this fact during a recent visit to Sweden. Several grasses, which are moderately susceptible to the avenae form of rust in the United States, but which seldom rust heavily in the field, were very generally and severely infected in Sweden. The fact that there are tremendous numbers of barberry bushes in some oat-growing regions of Sweden, and the additional fact that oats are so commonly grown might account for the abundance of the rust. In addition, however, Eriksson evidently was correct in assuming that the stem rust of oats was especially virulent in Sweden. This emphasizes still more the fact that the specialization of *P. graminis avenae* may be different in different countries. The present results indicate that the forms may also differ even in various regions of the same country. On the other hand more than one biologic form may occur in the same locality or even on the same plant.

While the virulent Swedish form of stem rust of oats may possibly exist in the United States, fortunately it does not seem to be either abundant or widespread in those Northern States in which White Tartar is commonly grown. The work of Durrell and Parker (1) indicates also that White Tartar is quite generally resistant to stem rust.

The practical significance of the existence of several biologic forms of *Puccinia graminis* on oats in the United States will depend on the number and the virulence of forms which may be found in the future. This problem is now being thoroughly investigated.

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## PLATE 1

Classes of host reaction: Resistant (R), susceptible (S), and intermediate (X), as indicated by different types of infection (0, 1, 2, 3, 4, and X) produced by biologic forms of stem rust on different varieties of oats.

Class R (resistant), including 3 subclasses corresponding to infection types 0, 1, and 2.

A.—Practically immune (0). No uredinia are developed, but sharply defined hypersensitive flecks are usually present.

B.—Extremely resistant (1). Infection very light; uredinia minute and scattered and surrounded by very sharply defined necrotic areas.

C.—Moderately resistant (2). Infection light; uredinia usually small and scattered; hypersensitive areas varying from sharply defined necrosis to pronounced chlorosis.

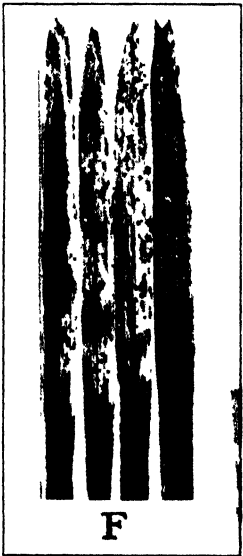
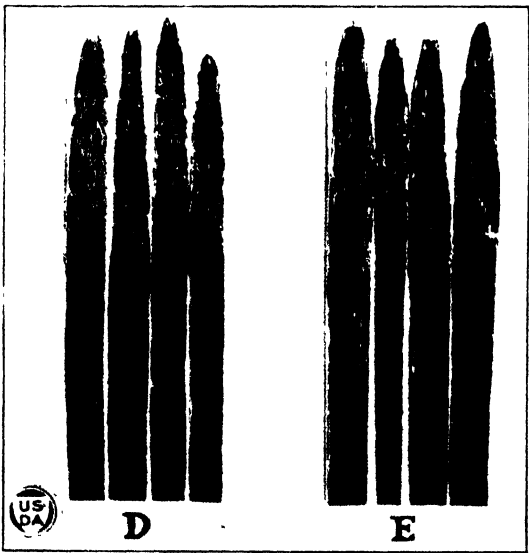
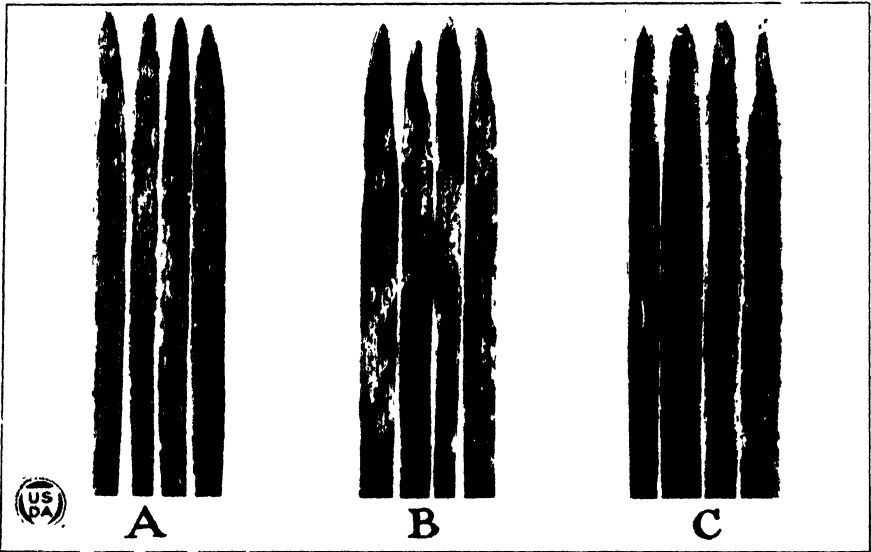
Class S (susceptible), including two subclasses corresponding to infection types 3 and 4.

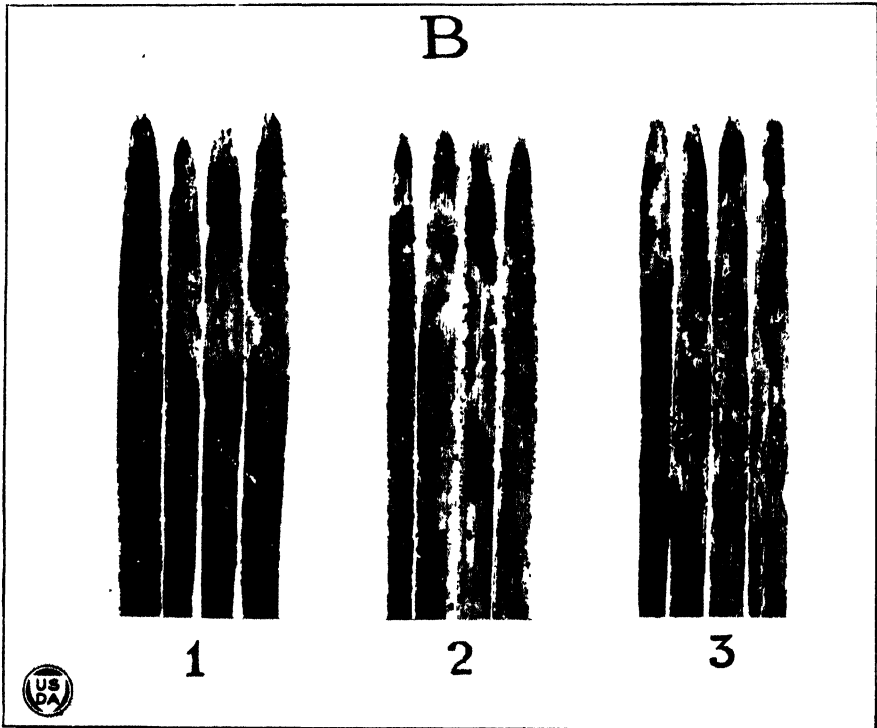
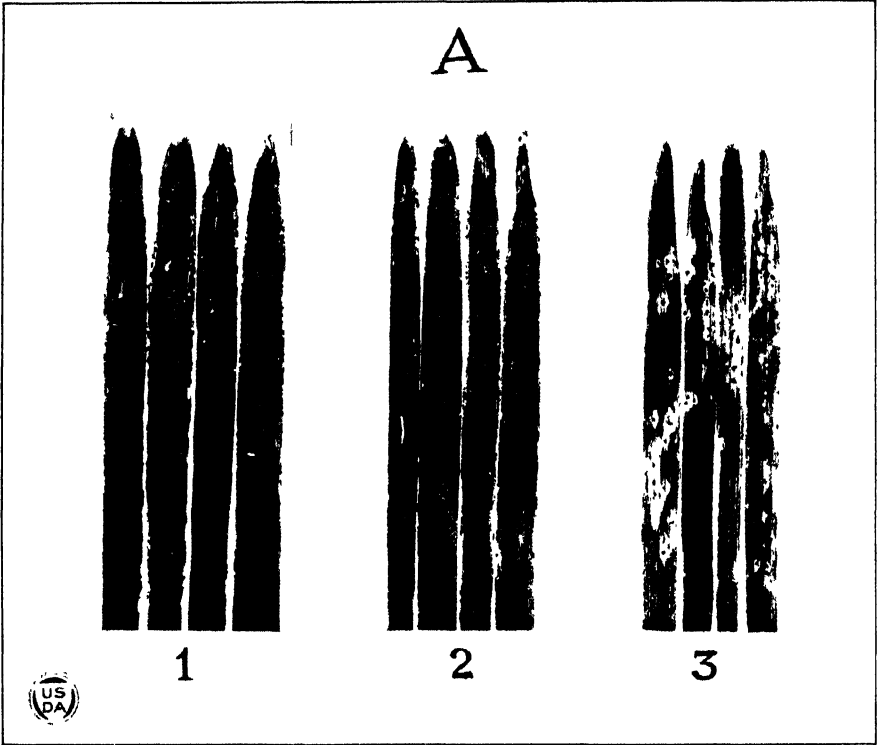
D.—Comparatively susceptible (3). Infection moderate; uredinia midsized with a tendency to coalesce; true hypersensitiveness absent, but light chlorotic areas usually present.

E.—Completely susceptible (4). Infection normal and heavy; uredinia large, numerous and confluent, hypersensitiveness entirely absent, but chlorosis may be present when cultural conditions are unfavorable.

Class X (intermediate), representing the heterogeneous (X) type of infection. (No subdivisions of this class are recognized.)

F.—Uredinia very variable, apparently including all types and quantities of infection, often of the same blade; no mechanical separation seems to be possible, as, on reinoculation, spores from small uredinia may produce large ones, and vice versa. In general, the infection is ill-defined.





**PLATE 2**

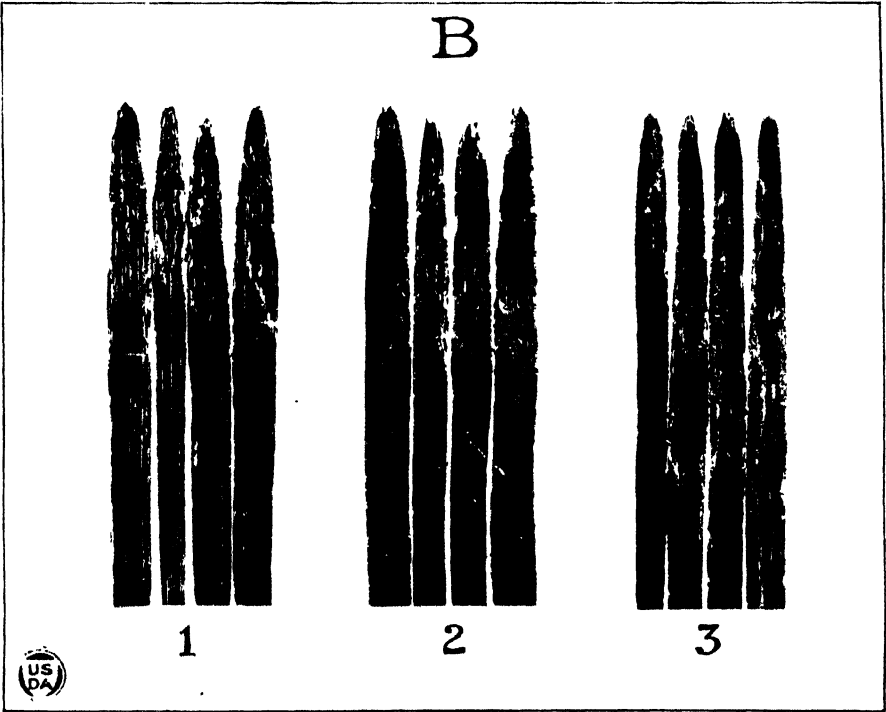
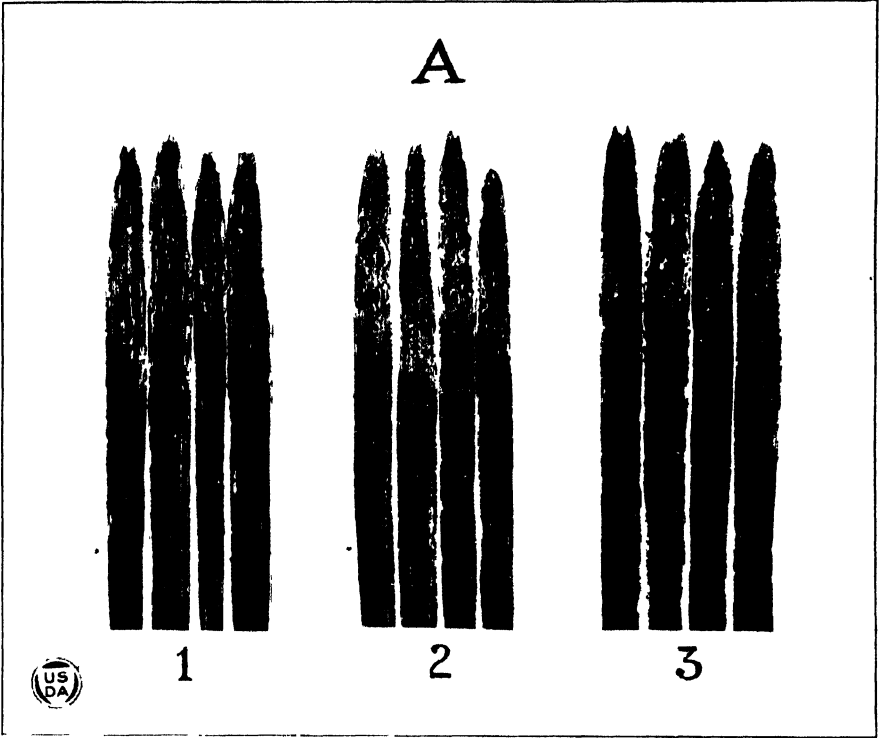
**A.**—*Puccinia graminis avenae* (Form I): (1) Victory, very susceptible; (2) White Tartar, quite resistant; (3) Monarch Selection, practically immune.

**B.**—*Puccinia graminis avenae* (Form II): (1) Victory, very susceptible; (2) White Tartar, quite resistant; (3) Monarch Selection, highly susceptible.

### PLATE 3

A.—*Puccinia graminis avenae* (Form III): (1) Victory, completely susceptible; (2) White Tartar, moderately susceptible; (3) Monarch Selection, very susceptible.

B.—*Puccinia graminis avenae* (Form IV): (1) Victory, completely susceptible; (2) White Tartar, highly susceptible; (3) Monarch Selection, very susceptible.





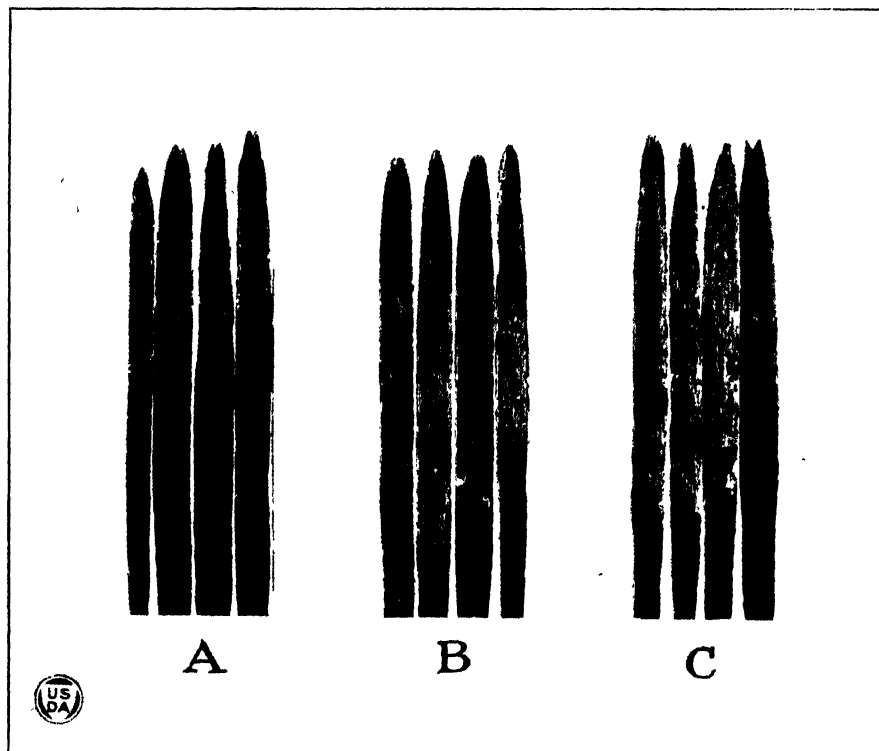


PLATE 4

*Puccinia graminis avenae* (Form V)

- A.—Victory, susceptible.
- B.—White Tartar, resistant.
- C.—Monarch selection, intermediate.



# DISEASE RESISTANCE TO ONION SMUDGE<sup>1</sup>

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## INTRODUCTION

In an earlier paper on onion smudge (24)<sup>3</sup> a description of the symptoms and seasonal development of the disease, and of the morphology and life history of the causal organism is given. In connection with the above studies it was found in confirmation of the earlier observations of Berkeley (1), Thaxter (18, p. 163-165), Halsted (8), and Selby (16, p. 364-366, 414), that all white varieties of onion observed are highly susceptible to smudge while varieties with colored bulbs—yellow and red—show a high degree of resistance. Further study as to the nature of this resistant quality has shown that there are at least two substances or groups of substances within onion tissue which have a marked inhibitive effect upon the growth of the causal fungus, *Colletotrichum circinans* (Berk.) Voglino. One of these is the volatile oil,<sup>4</sup> and the other is a group of one or more compounds closely associated or identical with the flavone and anthocyan pigments of the scales. The present paper contains the results of investigations upon this subject.

## OCCURRENCE OF VARIETAL RESISTANCE AND SUSCEPTIBILITY TO ONION SMUDGE

Observations on the relative susceptibility or resistance of onion varieties to smudge in field and storage have been continued since 1914, chiefly in Wisconsin and Illinois but incidentally in a number of other onion-growing sections in the United States. In 1922, the opportunity was given to secure field data from certain onion centers in Europe and the Canary Islands. In all cases there, as in this country, where colored and white varieties were grown simultaneously under conditions favorable for the development of smudge, white bulbs were uniformly infected while colored bulbs were completely free or infected to only a slight degree.

It should be pointed out in this connection that the pigment in colored varieties of onion appears in the outer bulb leaves or scales when the plants are about half grown, or earlier. Several of the first leaves of the plant acquire no basal thickness and the first of these slough off, without the production of much pigment, during the plant's early growth. With age the coloring becomes more intense until a short time before maturity,

<sup>1</sup> Accepted for publication Feb. 20, 1923. This study has been supported jointly by the Department of Plant Pathology, University of Wisconsin, through a special grant from the general research fund of the university, and by the Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

<sup>2</sup> The writer is indebted to Prof. L. R. Jones of the University of Wisconsin and to others in both the university and the Department of Agriculture for valuable suggestions and criticisms during the progress of the investigation.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 1038-1039.

<sup>4</sup> Throughout this paper the term "volatile oil" is used to designate the volatile substances which ordinarily arise from onion tissue, some of which, at least, are responsible for the characteristic onion odor.

when the yellow or red pigment, depending on the variety, is fully developed. At this time the plants have one to three thin, papery outer scales which are intensely colored. These cover the succulent scales and extend up over the neck of the bulb, where they frequently connect with their respective green portions of the tubular leaves or "top," although the latter may have disappeared by this time. Following these outer scales upward from the base, we find that the pigment ceases very abruptly above the bulb, leaving the neck sheathed largely by uncolored tissue (Pl. 1). Toward the end of the growing season there is among colored varieties a tendency toward some pigment reduction in the outer scales, especially in the portions above the soil line. This becomes more noticeable when bulbs are allowed to remain in the ground for a prolonged period after they are mature and ready for harvest.

When the disease appears early, several weeks before harvest, a few fruiting bodies of the fungus are occasionally found on the dead outer scales of colored varieties. These scales, usually only slightly pigmented, slough off before harvest. Practically all of the subsequent infection on colored varieties is confined to the outer sheath at or just above the neck of the bulb. This tissue, like the lower portions of the outer scale, is practically dead previous to harvest, but little or no pigment is present. This condition is illustrated in Plate 1, which shows one set each of red, yellow, and white varieties, all taken from the same field a few days before harvest. It is to be noted that the disease has become general on the bulb of the white variety, while in the yellow and red varieties it is confined to the uncolored sheath of the neck. In onion-set fields, this condition of infection at the neck very often becomes general on colored varieties if there is sufficient rainfall and high relative humidity to bring about dissemination of the fungus and infection. The intensely colored portions of the outer scales rarely show signs of the disease. The infection at the neck is largely sloughed off at harvest, leaving little or no sign of the disease in storage. On white varieties, as stated above, the fungus usually covers the entire bulb and continues to invade the fleshy scales, causing a gradual shrinkage.

#### INOCULATION EXPERIMENTS WITH BULBS

The field observations reported above have been confirmed by plot experiments in which the plants were grown on well-infested soil or were inoculated three to four weeks before harvest with spore suspensions of the causal organism. In these experiments a number of varieties of each color were included. Table I gives a list of the varieties used and the dates of the trials. These trials confirm the general field data in that the white varieties all appeared to be highly susceptible, while the colored varieties were highly resistant to the disease. In the case of the latter group, where infection did occur it was present in small amounts and usually at the neck of the bulbs where the pigment was less intense. Occasionally the outer pigmented scales bore some fungus stromata, but usually the latter were associated with a lessening of pigment due to one cause or another. In other instances, invasion of the fleshy scales was noted where they were exposed to the soil, due either to splitting or to partial disintegration of outer scales when the bulbs were not harvested promptly. This observation is in accord with the following experimental evidence which shows that when the fungus is applied directly to the fleshy colored scale, invasion readily takes place.

TABLE I.—List of onion varieties tested as to their susceptibility or resistance to smudge

Color.	1916	1917	1920	1921
White.	White Globe. White Portugal. Queen. Mammoth Silver King.  Extra Early Barletta. Giant White Italian Tri- poll.	White Globe.  White Portugal. Queen. Giant White Italian Tripoli.	White Globe.	Crystal Wax Ber- muda. White Australian. White Portugal. Early White Queen.
Yellow or brown.	Southport Yellow Globe. Michigan Yellow Globe. Yellow Globe Danvers. Yellow Danvers. Yellow Strassburg. Prizetaker. Early Neapolitan Marzola. Australian Brown.	Southport Yellow Globe. Michigan Yellow Globe. Yellow Globe Danvers. Yellow Strassburg.	Yellow Globe.	Ailsa Craig. Gigantic Gibraltar. Australian Brown.
Red.	Extra Early Red. Large Red Wethersfield. Southport Red Globe.	Extra Early Red. Large Red Wethersfield.	Red Globe.	Extra Early Red. Red Bermuda.

The inoculation studies were continued in moist chambers, where drops of spore suspension were placed directly upon marked areas of the surface of the scales. From certain of the bulbs the dry, outer scales were removed and the inoculum placed directly upon the succulent scale, while in others the natural conditions were simulated by placing the drops upon the dry scale. In no case did any infection whatever occur upon the dry outer scales of colored bulbs; and where the spores were examined in the drops of inoculum no normal germination had occurred. In nearly all instances, however, inoculations in the same or similar moist chambers upon the succulent scales of bulbs from the same original source did take effect. The infected area was characterized first by a very rapid disappearance of the pigment, often within two days after the inoculations were made, followed by a gradual collapse of the epidermal and underlying cells (Pl. 2, A). From all appearances the fungus invaded the so-called resistant varieties as aggressively as it did the susceptible ones when the dry scales were removed. The outer scales, therefore, appear to serve as the barrier and to be responsible for the resistant property of the colored varieties.

The rapid destruction of the coloring matter in succulent scales at first was very surprising in view of the marked toxicity of water extract of pigmented scales, which will be described later. In fact, it necessitated a further study of fungus invasion of the colored succulent scales. This was done by cutting tangential razor sections from the surface of the scale directly beneath the drop of inoculum so as to contain the undisturbed epidermis with a few layers of the underlying cells. These sections were then examined in water mounts under the microscope. By this method the progress of the fungus could be watched from the instant of spore germination throughout the disappearance of the pigment. Scales of a red variety were used because it was much easier to follow the latter phenomenon in them than in those of the yellow varieties. It was found that the process of invasion was exactly similar to that already described for white onion bulbs (24). After the formation of the appressorium and of the penetration tube, the mycelium developed first between the cuticle and sub-epidermal wall. Many cells were to be

found in which penetration of the cuticle had occurred with no apparent reduction of pigment. However, before the hyphae had developed to any great extent there was evidence of pigment reduction, which proceeded quite rapidly. From the evidence so far obtained it appears that the pigment was destroyed while the mycelium was developing in the outer wall and before actual penetration of the cell occurred. If this be true, the hyphae do not actually come into contact with the pigment as such; hence even if the latter were toxic, it might have no effect upon the fungus. The destruction of the pigment is due either to a substance secreted by the hyphae and capable of diffusing in advance of them or to independent chemical changes in the host cell brought about by the disturbances resulting from fungus invasion of the outer wall.

Of interest in this connection is the common observation made during these experiments—namely, as the lesion developed on the succulent red scales, pigment of normal intensity appeared in the lumina of several layers of the uninvaded parenchyma cells below and around the lesion. In normal, healthy scales these cells never contain pigment, the latter being confined to the cells of the outer epidermis. The significance of the appearance of pigment under these adverse conditions can not be entirely explained, but it seems to show very conclusively that the fungus infection at a given point on the scale not only affects the invaded cells but also influences either directly or indirectly the metabolism of uninvaded cells for some distance around the lesion. The stimulation of anthocyan production by fungus invasion is a matter of common observation in nature (25).

#### VARIETAL SUSCEPTIBILITY OF ONION SEEDLINGS

The writer has reported (24) experiments in which *Colletotrichum circinans* caused a damping off of White Globe seedlings under greenhouse conditions. It was of interest to note whether young seedlings of colored varieties would show any signs of resistance, especially since no pigment would have developed at this early stage. Sterilized greenhouse soil was inoculated with a pure culture of the organism, and seeds of a few of the varieties listed above were planted. The percentage of diseased plants in the white varieties were: White Portugal, 85 per cent; Giant White Italian Tripoli, 98 per cent; and Queen, 93 per cent; in the yellow varieties: Michigan Yellow Globe, 100 per cent; and Southport Yellow Globe, 93 per cent; and in the red varieties: Extra Early Red, 81 per cent; and Southport Red Globe, 95 per cent. The essentially equal susceptibility at this stage of all varieties tried, colored and white alike, is self-evident.<sup>5</sup>

The experimental and observational evidence conclusively shows that we have in colored onions a high degree of resistance to smudge but that this character is not present or at least not effective in the young seedlings. As will be shown later, the active resistant principle is a water soluble substance in the dry outer colored scales. This resistance is not to be construed as a case of absolute immunity nor is it unaffected by environmental conditions. This idea is substantiated by the fact that

<sup>5</sup> Under field conditions in our Northern States, the young onion seedlings, being spring-sown, are ordinarily growing in a soil too cool for the development of smudge. This probably accounts for the total lack of any report in America of damping off in the field due to the smudge fungus. In England (1922), however, this organism was found causing a damping off of young seedlings sown in August, at a time when soil temperatures were more favorable. This observation was on White Lisbon variety; no seedlings of colored varieties were available for comparison.

slight infection may occur on colored bulbs and even in rare occasions to a considerable degree if they are unduly exposed after maturity to the bleaching effects of direct sunlight as well as meteoric and soil water.

#### RELATION OF ONION OIL TO THE PARASITISM OF COLLETOTRICHUM CIRCINANS

The amount of research upon the chemical composition of the onion is limited. According to Remington and Wood (15, p. 1525-1526):

Fourcroy and Vanquihn obtained from the ordinary onion a white acrid volatile substance containing sulphur, albumen, much uncrystallizable sugar and mucilage, phosphoric acid both free and combined with lime, acetic acid, calcium citrate, and lignin. The expressed juice is susceptible of vinous fermentation. The oil is essentially the same in chemical composition as the oil of *Allium Sativum* and consists largely of allyl sulphide ( $C_3H_5$ )<sub>2</sub>S.

According to Gildemeister and Hoffman (7, p. 546), Semmler has found that onion oil, contrary to the reports given in most textbooks, consists not of allyl sulphid but of a series of sulphids, chiefly  $C_6H_{12}S_2$ . Kooper (11) found thiocyanic acid in the onion as well and secured positive tests for thiocyanic acid allyl ether, but no trace of acetic, formic, or allyl aldehyde. The germicidal effect of onion juice was noted by Pasteur (12).

#### RELATION OF EXPRESSED ONION JUICE TO COLLETOTRICHUM CIRCINANS

The relation of the constituents of the onion tissue to the fungus was first investigated experimentally by studying the effect of the expressed sap from onion scale upon spore germination. White onion scale tissue was macerated and the sap expressed by forcing through cheesecloth. Portions of the sap were diluted 10, 100, and 1,000 times with distilled water. Spores from a pure culture of the fungus were placed in drops of the expressed sap and in its various dilutions on glass slides in moist Petri dishes. No germination occurred in the sap as expressed from the tissue, nor in the 1 to 10 dilution. In the 1 to 100 dilution fair germination took place, and in the 1 to 1,000 dilution normal germination occurred. This experiment was repeated many times with practically the same results. When bits of fleshy onion scale tissue were added to drops of water containing spores, germination was also inhibited.

In order to determine the effect of heating on this inhibitive substance, a small amount of onion extract was secured in the usual way and divided into two equal parts, one of which was placed in live steam for 20 minutes. No germination occurred in the fresh extract as usual, while in the heated extract a high percentage of the spores germinated normally. From the results of these experiments it seemed quite certain that there was present in the extract a toxic substance which was readily removed by heat.

Since germination of the spore normally takes place in the soil or in water outside the plant, and not in contact with the host cell sap, and since the fungus would thus not come in contact with the inhibitive substance until its mycelium had penetrated the host, the question arose whether this toxic substance within the cell is capable of inhibiting the growth of the germ tube. Spores were germinated in distilled water until the germ tubes were several times the length of the spore, then a number of them were transferred to onion sap extract. The growth of the latter group was checked at once, while those left in water continued to grow at the normal rate. This showed that the mycelium could be checked by the



inhibitive substance of the cell sap. It seemed highly probable that this substance present in the living cells of the host was responsible, in part at least, for the checking of the advance of the fungus after it had penetrated the fleshy scales. It seems logical, therefore, to conclude that the restricted parasitism of the fungus is not due entirely to substances lacking in the fungus, but perhaps in some measure to the toxicity of the cell sap of the host.

#### EFFECT OF THE VOLATILE ONION OIL ON THE FUNGUS

Experiments were next undertaken to determine the effect of the volatile oil of the onion upon the germination and growth of the fungus. For the germination studies clean glass slides were laid in Petri dishes, two slides in each dish. The expressed juice of white onions, diluted to various degrees as in the above experiments, was used. Two drops of this medium containing the spores were placed on one slide in each Petri dish, while on the second slide in each dish were placed two drops of distilled water containing spores in suspension. The data secured from this experiment are tabulated in Table II.

TABLE II.—Effect of volatile oil of onion extract on germination of spores of *Colletotrichum circinans*

Petri dish No.	Slide No.	Medium.	Dilution.	Germination.	
				Experiment 1.	Experiment 2.
				Per cent.	Per cent.
1	1	Onion extract.....	Undiluted.	0	0
	2	Distilled water.....		0	0
2	3	Onion extract.....	1 to 10...	0	0
	4	Distilled water.....		75±	25±
3	5	Onion extract.....	1 to 100...	75±	25±
	6	Distilled water.....		75±	95±
4	7	Onion extract.....	1 to 1,000..	75±	95±
	8	Distilled water.....		75±	95±

The unusual and striking feature of these experiments is that in the Petri dishes containing the undiluted extract, no germination whatever occurred, either in the extract or in the drops of distilled water. This could be attributed only to the fact that a volatile substance from the expressed onion juice had been absorbed by the distilled water and had prevented the spores from germinating. The toxicity of the onion oil was thus clearly demonstrated. The toxic effect of the onion oil upon the spores in distilled water decreased as the onion extract was diluted. These experiments were repeated many times with essentially the same results.

The effect of the volatile oil was demonstrated in another way. A suspension of spores was made in two tubes of melted potato agar, which were then poured into sterile Petri dishes. After the agar had hardened the plates were inverted. In the center of the inside of the cover of one plate a small amount of onion extract was placed. The agar was thus exposed to the volatile oil, but was not in contact with the extract. The other dish served as a control (Pl. 2, B). In it the spores

germinated normally and growth soon covered the plate. In the other plate the volatile oil prevented germination of all the spores except those around the edge of the plate. In a few days the extract was decomposed by bacteria, since no attempt was made to keep it sterile; then the growth of the fungus proceeded from the margin toward the center of the plate. The fact that the spores in the center of the plate did not germinate showed that they had been killed by the volatile oil.

Another experiment was performed to determine the effect of the volatile oil on the growth of the mycelium. Three plates of potato agar were poured, and to the center of each was transferred a bit of mycelium from a pure culture of the fungus. The colonies were allowed to develop for four days, when they measured 18, 19, and 15.5 mm. in diameter, respectively. One plate was then designated as a control. Within the inverted covers of the other two were placed small amounts of onion extract as described in the previous experiment. In Table III are given the measurements of the colonies for the next five days. The colony in the control plate continued to grow at a normal rate, but those colonies exposed to the volatile oil practically ceased growth. At the end of the third day the extract was removed from one plate; the fungus colony in that plate resumed growth, showing that the mycelium had not been killed, but had merely been checked by the volatile oil. In the third plate a slight amount of growth had taken place by the fifth day, after which, as the onion extract decomposed, the fungus resumed the normal growth rate. This experiment shows that the growth of mycelium is markedly checked in the presence of the volatile oil, but that, unlike the spores, its growth processes are resumed when the inhibitive substance is removed. This fact is of significance in considering the effect of the onion oil upon the fungus hypha invading the scale tissue. One might expect in this case a slowing up or cessation of growth of the hypha, temporarily at least, but not necessarily a killing of the fungus filament.

TABLE III.—Effect of volatile oil from onion extract on growth of *Colletotrichum circinans*

Time of measurement.	Diameter of thalli.		
	Control.	Exposed.	Exposed.
	Mm.	Mm.	Mm.
Beginning of experiment.....	18	19	15.5
Second day.....	27	19	15.5
Third day.....	33	19	15.5
Fourth day.....	39	<sup>a</sup> 20	15.5
Fifth day.....	41	23	16.0

<sup>a</sup> Extract removed at end of third day.

#### ONION OIL, FROM RESISTANT AND FROM SUSCEPTIBLE VARIETIES

It should be kept in mind that the foregoing experiments were conducted entirely with extracts from white onions, which have been shown to be susceptible to the disease. It was of interest to know what degree of toxicity was to be found in the extract from the yellow and the red varieties, the so-called resistant types. Accordingly, extracts were made from Red Globe and Yellow Globe bulbs, as well as from the White Globe, a single bulb being used in each case. It should be pointed out

here that there is a certain amount of pigment present in the epidermal cells of the colored fleshy scales which was necessarily included in the extract. Moreover, it will be shown later that the water extract of dry pigmented scales is toxic to the fungus. It is believed, however, that in these fleshy scale extracts the pigments were so dilute as to have had little or no effect. Various dilutions of the fleshy scale extracts in distilled water were secured, as before, and their effect upon germination of spores was determined. As described in the previous experiment, a Petri dish containing one slide for drops of spores suspended in distilled water and one slide for spores in the onion extract, was used for each dilution. Results of this experiment are included in Table IV.

TABLE IV.—*Toxicity of succulent scale extract from White, Yellow, and Red Globe varieties of onion to the spores of Colletotrichum circinans*

Dilution.	Petri dish No.	Medium.	Spore germination. <sup>1</sup>					
			Experiment 1.			Experiment 2.		
			White.	Yellow.	Red.	White.	Yellow.	Red.
Undiluted...	1	Extract.....	o	o	o	.....	.....	.....
		Distilled water.....	o	o	o	.....	.....	.....
	2	Extract.....	o	o	o	.....	.....	.....
		Distilled water.....	o	o	o	.....	.....	.....
1 to 10.....	3	Extract.....	o	o	o	o	o	o
		Distilled water.....	++	++	++	⊖	⊖	o
	4	Extract.....	o	o	o	o	o	o
		Distilled water.....	++	⊕	++	⊖	⊖	o
1 to 20.....	5	Extract.....	.....	.....	.....	⊕	++	⊖
		Distilled water.....	.....	.....	.....	⊕	++	⊖
	6	Extract.....	.....	.....	.....	⊕	++	++
		Distilled water.....	.....	.....	.....	⊕	++	++
1 to 40.....	7	Extract.....	.....	.....	.....	++	++	++
		Distilled water.....	.....	.....	.....	++	++	++
	8	Extract.....	.....	.....	.....	++	++	+
		Distilled water.....	.....	.....	.....	++	++	+
1 to 60.....	9	Extract.....	.....	.....	.....	++	++	++
		Distilled water.....	.....	.....	.....	++	++	++
	10	Extract.....	.....	.....	.....	++	⊖	⊖
		Distilled water.....	.....	.....	.....	++	++	++
1 to 80.....	11	Extract.....	.....	.....	.....	++	+	⊖
		Distilled water.....	.....	.....	.....	++	++	++
	12	Extract.....	.....	.....	.....	++	+	⊖
		Distilled water.....	.....	.....	.....	++	++	++
1 to 100.....	13	Extract.....	++	++	++	++	++	⊕
		Distilled water.....	++	++	++	++	++	++
	14	Extract.....	++	+	++	⊕	++	⊖
		Distilled water.....	++	++	++	++	++	++
1 to 1,000....	15	Extract.....	++	++	++	.....	.....	.....
		Distilled water.....	++	++	++	.....	.....	.....
	16	Extract.....	++	++	++	.....	.....	.....
		Distilled water.....	++	+	++	.....	.....	.....

<sup>1</sup> Symbols used: o=no germination; ⊖=o to 25 per cent spores germinating; --=25 to 50 per cent; ⊕=50 to 75 per cent; ++=75 to 90 per cent; +++=90 to 100 per cent.

It is quite evident that but little difference exists between the colored and white Globe varieties used as to the toxicity of their cell sap. In fact, in this respect the differences between individual bulbs of the same

variety are as great as those between varieties. It is to be expected, therefore, that the volatile oil content of these varieties of onions is a factor not necessarily responsible for differences in susceptibility or resistance to smudge.

From the foregoing experiments it may be concluded that the expressed sap from white onion scale inhibits germination and growth of *Colletotrichum circinans* and that this toxicity appears to be due, in large measure at least, to the volatile onion oil which is liberated upon crushing of the tissue. This toxic substance is thermolabile inasmuch as it is largely removed by heating the extract for 20 minutes in live steam. The fact that no appreciable difference in toxicity between sap from colored and that from white Globe scales was found indicates that the volatile oil has little to do with the difference in varietal susceptibility. It is believed, however, that this substance is not to be overlooked as a factor in limiting the parasitism of *C. circinans*.

It is of interest in this connection to note that Bernard (2) found the bulb tissue of *Loroglossum* to have a fungicidal effect upon the mycorrhizal fungi isolated from a number of closely related species of orchid. This was demonstrated by placing a piece of the bulb on agar in proximity to the thallus of the fungus. Growth of the latter did not extend within a certain zone surrounding the bit of orchid tissue.

#### RELATION OF SCALE PIGMENTS TO DISEASE RESISTANCE

It will not be necessary to review the literature upon plant pigments since it has already been brought together by others, including Wheldale (25) and Wakeman (22). Although a few cases of correlation between resistance and the red or yellow pigments in plants have been noted (5, 6, 17, 21), in no previous case, so far as the writer is aware, has the relation of plant pigments to plant parasites been carefully studied. The fact that a water extract from pigmented onion scales has been proved to be highly toxic (23) to the smudge organism, has prompted a further inquiry into this subject. It is needless to point out that the widespread occurrence of closely related pigment compounds in the plant kingdom justifies further investigation of their relation to parasitism.

#### NATURE OF THE RED AND YELLOW PIGMENTS IN ONION SCALES

The red and yellow pigments of onion scales are solutes in the cell sap of the outer epidermal layer. They first appear when the plants are about half grown and continue to develop during the remainder of the growing season. At maturity the color is most intense in the outer scales which have dried down to a thin papery consistency, and it becomes progressively less marked in the fleshy scales as the center of the bulb is approached. Intensity and color of pigment vary with varieties. Colored varieties may be roughly grouped into the large classes, the yellow and the red. There are numerous varieties, however, such as the Australian Brown, which lie on the border line between the two groups. Pigmented cells of the yellow varieties when treated with alkalis turn deep brownish yellow in color, a reaction typical of the flavones (25). The pigmented cells of the red varieties turn pink in acid and green in alkaline solutions, which reactions are characteristic of the anthocyanins. Perkin and Hummel (14) isolated quercetin, a flavonol,

from a hot water extract of colored onion scales, but they did not state the variety or color of onion used. Perkin and Everest (13, p. 201) state that attempts to isolate a quercetin glucoside from onion skins have hitherto failed. Our work shows that quercetin exists to a considerable extent in both the yellow and the red scales, and in the last case it evidently occurs along with an anthocyan. Beyond the work of Perkin, just cited, there is no information available as to the exact nature in which the red and yellow pigments exist in the onion tissue. In connection with the present problem this question is now being investigated in cooperation with the Department of Pharmacy, University of Wisconsin, and results with their bearing upon the relation between parasite and host will be published later. It is a matter of common observation that upon death of the cell, the cell sap pigments readily diffuse out. Likewise in the onion, there is considerable diffusion of soluble pigment from dead outer scales but little or none from the succulent scales.

#### RELATION OF ONION PIGMENTS TO THE FUNGUS

The close correlation between presence of pigment in the outer scale of the onion and resistance to smudge has already been pointed out. In further search for the substances in the plant responsible for resistance, a study was made of the effect of water extracts of the dry outer scales upon the germination and growth of the fungus.

It has been shown that when bits of the fleshy scale of the white or colored onion were placed in drops of water containing spores of *Colletotrichum circinans*, germination was entirely inhibited. Similar trials made with outer dead scales of white onion yielded no inhibition, however. Thus, it was evident, that a large part of the volatile oil present in the fleshy tissue is lost as the outer scale dries down. It is assumed, therefore, where dry outer scales were used that toxicity of the volatile oil did not enter in to any appreciable degree in the following experiments.

#### METHODS

The methods used were as follows: Drops of distilled water containing spores of the fungus were placed on clean glass slides, two drops on each slide. Petri dishes lined with moistened filter paper were used as moist chambers; two slides were placed in each Petri dish, one serving as a control, the other as a means of testing the effect of scale extract on spore germination. In the drops on the latter slide bits of onion scale about 2 mm. square were placed, usually two such pieces per drop. Observations were made and recorded after 18 to 24 hours.

#### EFFECT UPON SPORE GERMINATION

Further experiments, conducted with bits of tissue from the dead outer scales, resulted in quite a different reaction on the part of the fungus than had hitherto been noted. No indication of the injury to control drops so evident in the last experiment was noted, showing that the fungicidal effect of the volatile oil is largely, if not entirely, absent in the dry outer scales. In the case of drops of spore suspension containing bits of the dry white scale, germination occurred and appressoria formed as usual. The behavior of the organism was thus very

similar to that commonly observed in distilled water or on nutrient media. However, where red or yellow scale tissue was added to the drops distinctly abnormal germination occurred. In a large majority of cases, the spore sent out a germ tube, which usually acquired a length of only about one micron, when the wall at its tip either dissolved or ruptured and a portion of the cell contents exuded and collected in a naked mass at the end of the very short and usually indistinguishable tube. Thus at the end of 12 to 18 hours most of the spores were to be found each with a naked mass of the cell contents adjacent to them. This feature is illustrated in figure 1 and Plate 3. For the purpose of the present paper this condition is referred to as "rupturing" of the germ tube, although the exact details of the process have not been worked out and it is realized that further study may necessitate another descrip-

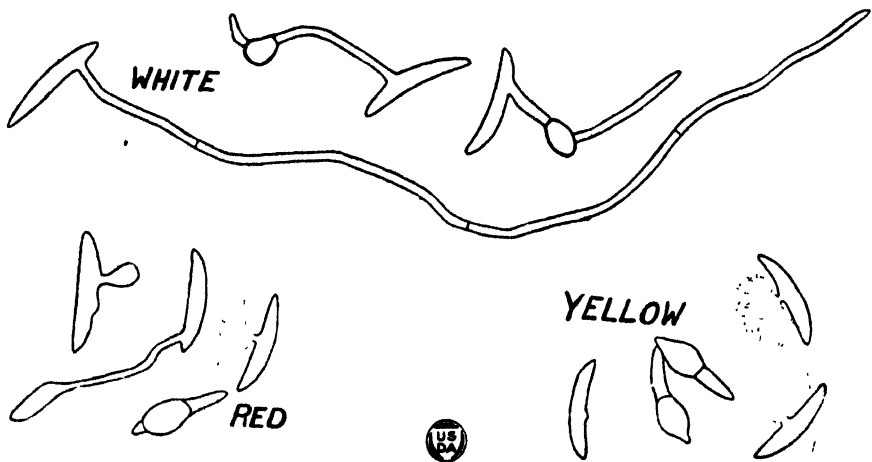


FIG. 1.—Effect of water extract of outer onion scales upon spore germination of the smudge fungus. Bits of dry scales of white, yellow, and red onions were added to drops of spore suspension in distilled waters. Note typical germ tubes and appressoria in the case of the white scale as compared with abnormal germination in the case of the colored scales. The abnormalities are of three types: (1) Ruptured germ tubes, (2) short, thick germ tubes, (3) swelling of spores without production of germ tubes.

tive term. This phenomenon usually prevented further functioning of the spore.

In a smaller percentage of cases there occurred other abnormal types of reaction, which may be divided into two general classes. The first class includes those which will be referred to as "swollen spores." In this group there is a marked swelling of the spores, usually at one end, with septation occurring at the limit of this enlargement (fig. 1); no germ tubes are formed. In the second class, designated as having "abnormal germ tubes," the tube grows for a short distance, is usually swollen at the tip or abnormally large in diameter, and never produces an appressorium (fig. 1).

The percentages of various types of germination obtained in the first experiment are recorded in Table V. The results were determined by counting several microscopic fields from each slide. It is to be noted that ruptured germ tubes occurred with a large majority of the spores where red or yellow scale tissue was added to the drops. Moreover, the spores reacted essentially alike to the red and yellow tissue. Numerous repetitions of this experiment yielded essentially similar results. The data from one of these experiments are recorded in Table VI.

TABLE V.—Effect of dry outer scale tissue of red, yellow, and white varieties of onion upon the spore germination of *Colletotrichum circinans* (experiment 1)

Variety.	Germination.				
	No germination.	Normal germination.	Ruptured germ tubes.	Abnormal germ tubes.	Swollen spores.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Red.....	0	21	78	0	1
Yellow.....	0	35	61	2	2
White.....	10	90	0	0	0

TABLE VI.—Effect of dry outer scale tissue of red, yellow, and white varieties of onion upon the germination of *Colletotrichum circinans* (experiment 2)

Petri dish No.	Color of tissue used.	Germination.				
		No germination.	Normal germination.	Ruptured germ tubes.	Abnormal germ tubes.	Swollen spores.
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
1	Red.....	7	2	45	42	4
	Control.....	9	91	0	0	0
2	Red.....	12	0	88	0	0
	Control.....	12	88	0	0	0
3	Yellow.....	34	0	66	0	0
	Control.....	7	93	0	0	0
4	Yellow.....	24	0	76	0	0
	Control.....	11	89	0	0	0
5	White.....	8	92	0	0	0
	Control.....	9	91	0	0	0
6	White.....	13	87	0	0	0
	Control.....	47	53	0	0	0

## EFFECT UPON GROWTH

The experiments just described show conclusively that the water extract of pigmented dry scales is highly toxic to spores of *Colletotrichum circinans*. while a similar extract from white scales has no such inhibitory effects. The fact that the toxic substance (or substances) is so readily diffusible leads one to surmise that the plant may be protected by its gradual solution into the soil water immediately surrounding the bulb. The smudge organism develops normally in the soil; hence it is conceivable that in some cases at least the spore may germinate at some distance from the bulb; thus the mycelial hyphae may be the first to come into close contact with the bulb or with the soil solution immediately surrounding it. Accordingly an experiment was carried out to determine the effect of the pigmented extract upon the growth of the fungus mycelium.

Spores were germinated in water until the germ tubes were several times the length of the spores. Small pieces of dry outer red scales were then added to certain of the drops, while others were left as controls. After 24 hours, examination showed that whereas the hyphae in the control drops had continued to grow normally, those to which the bits of red scale had been added had grown little if at all from the time these pieces

were added. This is best shown in figure 2, which contains camera-lucida sketches of representative spores and thalli, made at the time when the scale tissue was added to the drops of spore suspension and at the conclusion of the experiment. It is quite evident that the toxic substance (or substances) here concerned inhibits the growth of hyphae as well as the normal germination of spores of *Colletotrichum circinans*.

#### EFFECT OF COLORED AND OF UNCOLORED PORTIONS OF THE SAME SCALE

It has already been pointed out that ordinarily little or no pigment is formed in the outer scales at the neck of the bulb. Moreover, under suitable conditions this particular portion of an otherwise resistant plant is readily attacked. It was important, therefore, to know whether the inhibitive substance already demonstrated in the pigmented tissue could be found in this closely adjacent uncolored area. The outer scale of a red onion set (Red Wethersfield variety) which had become naturally infected in the uncolored portion at the neck was carefully removed.

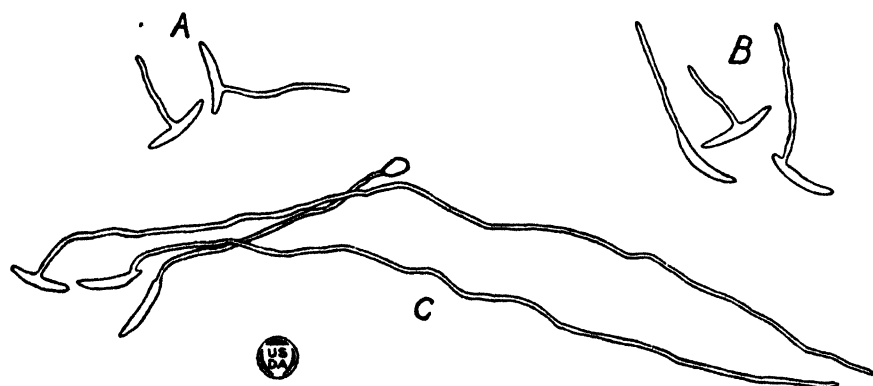


FIG. 2.—Effect of colored outer scale extract upon growth of hyphae of the smudge fungus. Spores were germinated in drops of distilled water to the point illustrated by camera lucida sketches in A. To some of the drops bits of red outer scale were then added. Growth was recorded by further sketches 24 hours later. In B are shown typical spores from drops to which red scale tissue has been added, while in C are shown typical spores from control drops. Growth was almost, if not entirely, checked when the scale tissue was added.

Bits of tissue were then cut from the colored part of the scale just below the area of infection, and from the uncolored part about 1 inch above this point. The scale after the removal of these bits of tissue is shown in Plate 4. The bits of tissue from the two points were placed in drops of spore suspension according to the previously described method. In the drops containing uncolored tissue normal germination occurred, while in those containing colored tissue practically all of the spores developed ruptured germ tubes. Representative spores from each lot are illustrated in Plate 4. The experiment has been repeated several times with similar results, showing conclusively the close correlation existing between the pigment and the toxic entity.

#### EFFECT OF DILUTING THE PIGMENT EXTRACT

In order to secure a more nearly uniform basis of comparison between white, yellow, and red bulbs, an equivalent amount of dry scale tissue from each variety was weighed. The three lots were then allowed to steep for 24 hours at room temperature in distilled water added at the



rate of 20 cc. per gram of tissue. The extract was filtered and various dilutions of the filtrate with distilled water were used as media for spore

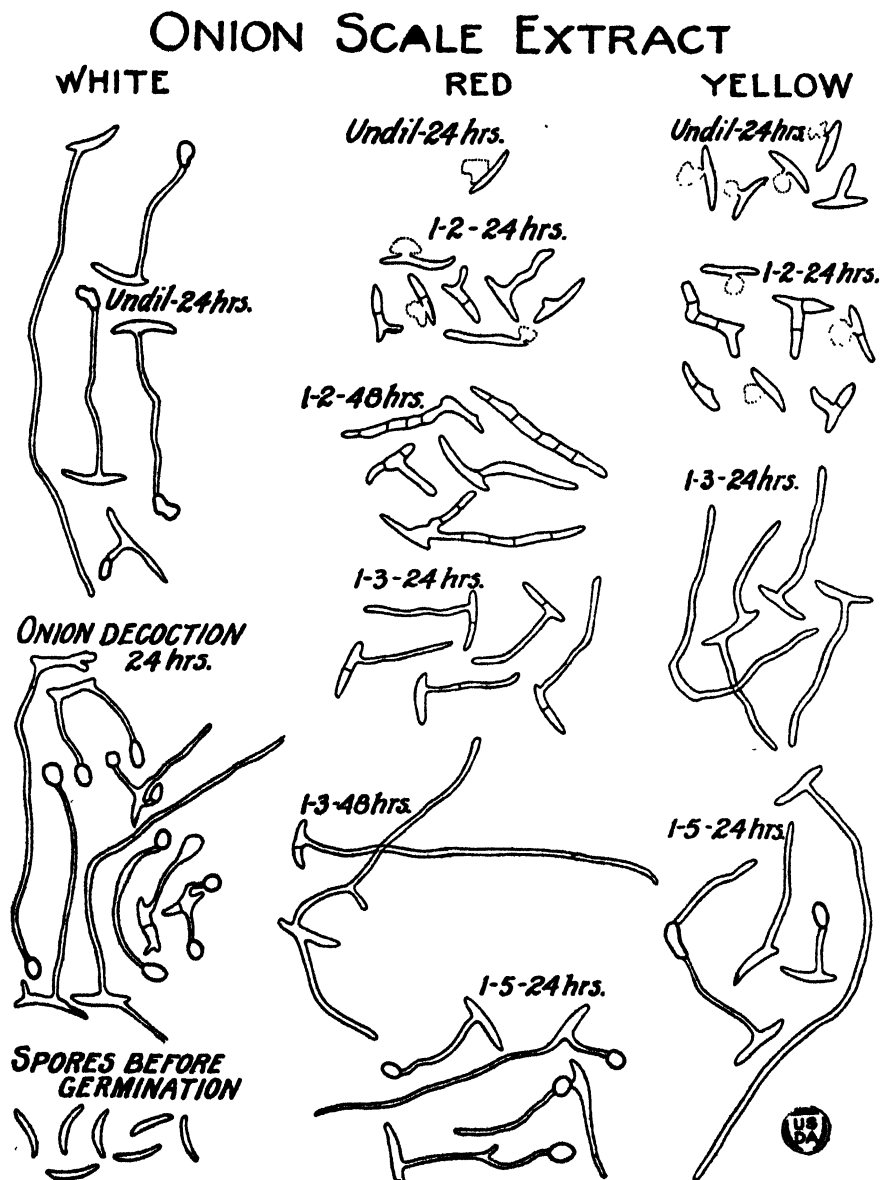


FIG. 3.—Spore germination of *Colletotrichum circinans* in extracts made from dry outer scales of white, red, and yellow varieties of onion, and in onion decoction. One gm. of dry scale was extracted over night in 20 cc. of distilled water at room temperature. Dilutions were then made up to 1 to 5 with distilled water. Note that germination and growth were normal in onion decoction and undiluted white scale extract, while in the undiluted colored scale extract typical abnormal germination occurred as shown in fig. 1. With dilution the toxicity of the extract was reduced, however, quite normal germination occurring at 1 to 5. Normal ungerminated spores are shown in the lower left-hand corner. See further explanation in the text.

germination. The results are recorded below; representative spores from each dilution are illustrated by camera lucida sketches in figure 3.

White scale tissue:

Undiluted—Good germination, growth, and appressorium formation.

## Yellow scale tissue:

Undiluted.....At 24 hours, a large percentage of ruptured germ tubes; at 48 hours, no change.

1 to 2.....At 24 hours, many ruptured germ tubes and many short, thick, germ tubes; at 48 hours, practically no change.

1 to 3.....At 24 hours, short, thick germ tubes, no appressoria; at 48 hours, good growth and appressorium formation.

1 to 5.....At 24 hours, good growth and appressorium formation.

## Red scale tissue:

Undiluted.....At 24 hours, very little germination; when it occurred it resulted in ruptured germ tubes; at 48 hours, no change.

1 to 2.....At 24 hours, ruptured and short, thick, abnormal germ tubes; at 48 hours, some additional growth of latter.

1 to 3.....At 24 hours, germ tubes slightly longer than in 1 to 2, no appressoria, an occasional ruptured germ tube; at 48 hours, considerable additional growth, but no appressoria.

1 to 5.....At 24 hours, good germination and appressorium formation.

It will be seen that in the undiluted extract of white scales, germination was quite normal and compared favorably with that in onion decoction. In the undiluted red and yellow scale extract "ruptured" germ tubes were common. As the colored extracts were diluted, however, there was a gradual diminution of the toxic effect with seemingly normal germination occurring in the 1 to 5 dilution. This shows that the inhibition in the concentrated solutions was not due to a lack of nutrients. Moreover, the toxic entity was apparently about equally concentrated in the extract from the red and that from the yellow scales, if we may judge from the extent to which germination was reduced in equal dilutions of the two extracts.

## RELATION OF CELL SAP ACIDITY TO THE FUNGUS

The reaction of the cell sap of the host plant as a factor in resistance to parasites has been emphasized by Comes (3, 4), but Jones, Giddings, and Lutman (10), Hawkins and Harvey (9), Vavilov (20), and others found no positive evidence of its importance as the cause of resistance. Since this point is not one to be overlooked, a study of the acid toleration of *Colletotrichum circinans* was made.

Onion decoction, a medium which had proved very favorable for germination and growth, was used in this study. Two hundred gm. of fresh onion bulb tissue and 1,000 cc. of distilled water were cooked in the steamer for one hour. This decoction was then filtered through filter paper and the hydrogen-ion concentration of the filtrate determined. The medium was then divided among ten 200-cc. flasks and the acidity of each adjusted with standard solutions of hydrochloric acid or with sodium hydroxid so as to cover the following range of  $P_H$  values: 1.8, 2.0, 3.0, 3.6, 4.4, 5.2, 6.4, 7.4, 8.8, and 9.4. A portion from each flask was used for spore germination tests and the remainder sterilized for 30 minutes at 7 pounds pressure. The acidity of each flask was again tested after sterilization and the following values noted: 1.6, 1.8, 2.2, 3.4, 4.6, 5.2, 6.2, 6.8, 7.4, and 8.2. Spore germination tests were also made with portions from these lots after sterilization. Good germination occurred in the alkaline media and in the acid up to  $P_H$  3.4. When the acidity increased beyond that point, germination was reduced and abnormal. In the unsterilized extract at  $P_H$  1.8 and 2.2, 10 to 15 per cent of the spores began to germinate, but the germ tubes appeared to rupture and exude cytoplasm in a manner strikingly similar to that observed in the water extract from dry

colored onion scales. Occasionally there was a slight swelling of the spores (fig. 4). At  $P_H$  3, about 43 per cent of the spores germinated, but the hyphae were abnormal in size and stunted, and in many cases the germ tubes ruptured. In the sterilized extract no germination occurred at  $P_H$  1.6 to 2.2, except for an occasional ruptured germ tube at  $P_H$  2.2.

The growth of the fungus on onion decoction of various  $P_H$  values was tested. The medium was made as described above, except that 2 per cent dextrose was added. Duplicate portions of 75 cc. each in 300-cc. Jena glass flasks were used. The medium was inoculated by transferring a piece of stroma and mycelium from a potato agar culture of the organism to each flask. The flasks were kept in diffused light at room temperature ( $20^\circ$  to  $25^\circ$  C.) for 54 days. The mycelial growth was then removed from the remaining culture medium by filtration and the reaction of the filtrate determined. The  $P_H$  value of the medium before and after filtra-

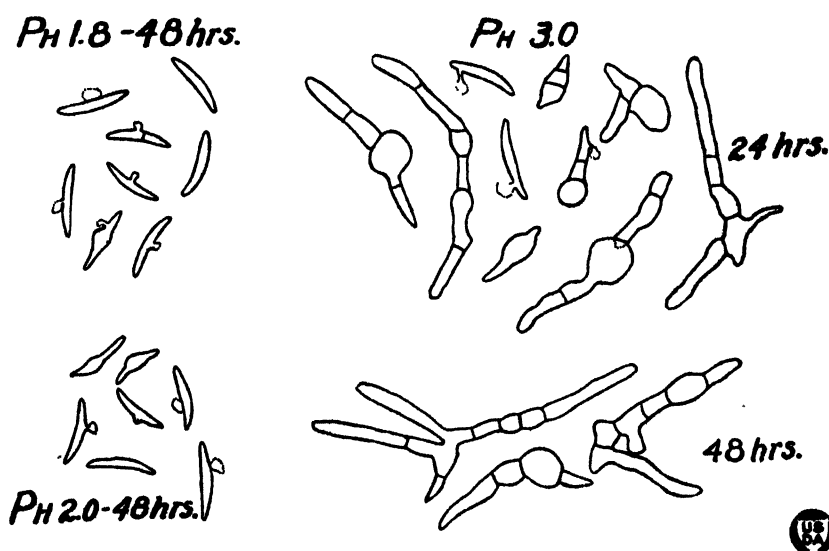


FIG. 4.—Spore germination of *Colletotrichum circinans* in onion scale decoction adjusted to the various degrees of acidity indicated. Note the ruptured germ tubes at  $P_H$  1.8 and 2.0, and the same together with swollen spores and short thick germ tubes at  $P_H$  3. See further explanation in the text.

tion, and at the end of the experiment, as well as the amount of fungus growth occurring in the flasks, are recorded in Table VII.

A repetition of this experiment with Pyrex flasks and the use of spores instead of mycelium as inoculum yielded essentially the same results.

It may be concluded from the above experiments that both the spores and the mycelium of *Colletotrichum circinans* germinate and grow well in onion decoction ranging in  $P_H$  value from about 3.5 to 8. As the actual acidity increases beyond 3.5 there is a sharp reduction in germination and growth, with a tendency toward rupturing of the germ tubes. It is important also to note that in the flask cultures, where good growth occurred, the reaction of the medium changed decidedly toward the alkaline during the period of the experiment. The rapidity of this change and the actual variations in the reaction from day to day were not determined.

The acid toleration of the fungus having been studied, the reaction of the onion tissue was next considered. Plants of the Red, Yellow, and White Globe varieties growing out-of-doors were pulled at about 8.30

a. m., July 31, 1920, and determinations made directly by the electrolytic method.<sup>6</sup> The tops and the scales were chopped up separately and the expressed juice used for the determinations. The results are given in Table VIII. It is to be remembered, of course, that this method does not necessarily give the actual reaction which the fungus hyphae encounter as they invade the plant. It is conceivable that there may be natural variations in reaction in different parts of the bulb and at different hours of the day, while secretions of the fungus itself might change its immediate surroundings. Valteau (19) suggests in the case of brown-rot of plums (*Sclerotinia cinerea*) that, "it is very probable that in the slow development of the fungus in the resistant fruits enough oxalic acid is produced by the hyphae to actually become toxic to them." However, there was no evidence of wide variations in acidity between resistant and susceptible onion bulbs, and the acidity of the juice expressed from either was much lower than that of the onion decoction in which germination and growth of the fungus were inhibited. Moreover, the fungus tended to change very acid solutions to a more alkaline, and thus more favorable, reaction.

TABLE VII.—Growth of *Colletotrichum circinans* in onion decoction adjusted to various  $P_H$  values (duration of experiment, 54 days)

Flask No.	$P_H$ value.			Amount of growth.
	Before sterilization.	After sterilization.	At end of experiment.	
1.....	2.0	1.6	1.8	None.
2.....	2.0	1.6	1.8	Do.
3.....	3.0	3.0	2.0	Do.
4.....	3.0	3.0	2.0	Do.
5.....	3.2	3.0	2.6	Very slight.
6.....	3.2	3.0	2.6	Do.
7.....	3.8	3.6	5.2	Good growth and sporulation.
8.....	3.8	3.6	4.8	Do.
9.....	4.6	4.6	6.2	Do.
10.....	4.6	4.6	6.2	Do.
11.....	5.2	5.4	7.2	Good growth.
12.....	5.2	5.4	7.0	Do.
13.....	6.4	6.4	7.4	Do.
14.....	6.4	6.4	7.4	Do.

TABLE VIII.—Acidity of expressed juice of scales and tops of Red, Yellow, and White Globe onions

Variety.	$P_H$ values.	
	Tops.	Succulent scales.
Red Globe.....	5.2	5.3
Yellow Globe.....	4.8	5.4
White Globe.....	5.2	5.4

<sup>6</sup> The writer is indebted to Mr. O. C. Bryan of the Department of Soils, University of Wisconsin, for making the hydrogen-ion determinations.

The possibility of a connection between acidity and toxicity in cold water extracts of dry outer scales was considered. Outer scales of red, yellow, and white bulbs were extracted for 24 hours in redistilled water having an approximately neutral reaction. For each gram of tissue 15 cc. of water were used. The hydrogen-ion concentration of each extract was then determined and portions of each were used for spore germination tests. The results are given in Table IX. Although the acidity of the outer scale extracts is somewhat higher than that of the succulent tissue, it was still in all cases within the range tolerated by the fungus, and the toxicity, therefore, must be due to something beside acidity. In none of these determinations was there any evidence of wide variation in acidity between resistant and susceptible bulbs.

TABLE IX.—Acidity of cold water extracts of dry outer scales of white and colored onions

Variety.	P <sub>H</sub> value of extract.	Reaction of spores.				
		Ungerminated.	Normal germination.	Ruptured germ tubes.	Swollen spores.	Short, abnormal germ tubes.
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Yellow Globe. ....	3.70	52	0	47	1.0	0.0
Yellow Globe. ....	4.67	24	0	74	1.0	1.0
Red Globe. ....	3.55	91	0	8	0.5	0.5
White Globe. ....	4.03	Percentage germination fairly high; good growth; numerous appressoria; new conidia being formed at 48 hours, making spore counts impossible.				

## DISCUSSION OF RESULTS

The resistance of the common colored varieties of onions to smudge is well established. The water extracts from the dry outer scales of red and of yellow onions have shown a marked toxicity to spores and mycelium of *Colletotrichum circinans*, and the reaction of the fungus is quite different from that brought about by the onion oil. In the scales of white varieties the toxic entity ordinarily associated with flavone or anthocyan pigments in the colored varieties, is apparently absent or too slight to be detected. Moreover, in the colored varieties, it is confined to that tissue in which there has been production of pigment. Its absence in the unpigmented portions of the colored scales about the neck of the bulb is correlated with a marked susceptibility to the disease in this limited region. The fact that this toxic substance is readily diffusible in water indicates that meteoric or soil water immediately adjacent to colored bulbs may become sufficiently toxic to ward off invasion by the fungus. In any case, we are led strongly to suspect that by means of this toxic substance the dry outer scales of colored bulbs serve as a barrier between the parasite in the soil and the underlying fleshy scales, which have been shown to be susceptible to infection.

The exact identity of the toxic substance (or substances) remains to be determined. Its very close association with the scale pigments is suggestive. The toxicity may be due to the color compounds or to compounds closely associated with them. Attempts are now being made to isolate the substance in pure form, but it must be recognized

that such an analysis breaks down to some extent the complex balance of compounds as they exist in the tissue.

The marked toxicity of the expressed juice of fleshy scales of both the susceptible and the resistant varieties is also of interest. It seems to be due largely to the volatile onion oil, but apparently it does not contribute to the noted differences in varietal susceptibility of the northern Globe types. The juice from colored scales and that from white scales are essentially equal in toxicity. Moreover, as stated above, infection of the fleshy scales seems to progress as readily in resistant as in susceptible varieties. The relation of the volatile oil to the aggressiveness of the parasite may well be considered, however. The smudge organism causes an exceedingly slow decay of the bulb compared with certain other bulb parasites, such as *Botrytis allii*, for instance, although penetration by the former is accomplished very readily and easily and in fact much more generally than in the case of the latter fungus, which usually requires a wound in order that invasion may take place. When we consider the marked toxicity of the onion oil to the smudge fungus, we are led to wonder that invasion occurs at all. However, several possible explanations at once present themselves. In the first place, the oil as it is released from the expressed sap may be in a different state than that in which it actually exists in the living cell. In the allied case of the mustard oil of the crucifers, allyl isothiocyanate, for instance, the oil exists in certain cells as the glucoside, sinigrin, while in adjoining cells is contained a glucoside-splitting enzym, myrosin. When the tissue is crushed the enzym comes into contact with the glucoside and the volatile oil is released. Does a similar condition prevail in the onion? In the second place, under ordinary natural conditions the mycelium of the smudge fungus apparently does not invade the living host cells, but weakens and kills them, usually slightly in advance of the hyphae, by means of some diffusible substance. Moreover, the change in color and texture of the cell contents and, in the case of red scales, the tendency toward formation of coloring matter in the parenchymatous cells both indicate certain effects on host metabolism ahead of the parasite. It may be that through these changes in the cells the volatile oil is either broken down or released and hence that its toxic effects are reduced, at least to the point where invasion is merely retarded and not entirely checked.

The study of penetration indicates that the fungus is capable of penetrating the cuticle and dissolving the cellulose of the walls equally well in all varieties. The acid range tolerated in culture media is wider than the range occurring in the host. It is assumed, therefore, that differences in cell membranes and in acidity of cell sap do not materially affect resistance to the smudge organism.

#### SUMMARY

(1) Onion smudge is a common disease of the bulb scales. In general, white varieties are susceptible to the disease while colored varieties show a high degree of resistance.

(2) When the dry outer scales of colored varieties were removed the fleshy scales were found to be very susceptible, invasion taking place in the same manner as in the white varieties. The highly colored dry outer scales apparently serve as a barrier to the entrance of the parasite.

(3) In the seedling stage all varieties tried were equally susceptible.

(4) The expressed juice of succulent onion scales is highly toxic to the fungus; this effect is seemingly due in large measure to the volatile onion oil. The extracts from resistant and from susceptible varieties have essentially equal degrees of toxicity. The onion oil apparently is not a factor contributing to varietal resistance to smudge in the northern Globe types, but its importance in limiting the parasitic action of the fungus is suggested.

(5) The red and yellow colors in onion bulbs are due to pigments which are present in the cell sap of the outer epidermal layer. This color is most intense in the dry outer scales, and at least a portion of the pigment in these is readily soluble in water.

(6) Water extract of dry outer pigmented scales causes abnormal germination and retards growth of the fungus, while that from dry outer white scales does not. Essentially the same reaction was recorded with the yellow as with the red scales.

(7) A substance (or substances) present in the dry outer scales of resistant bulbs and closely associated or identical with the red and yellow pigments is apparently the chief factor causing resistance to smudge. Studies are now being directed toward isolation and identification of the toxic entity.

(8) Within the range determined for host tissue, variation in the hydrogen-ion concentration of onion decoction as a medium affects neither germination nor growth of the fungus.

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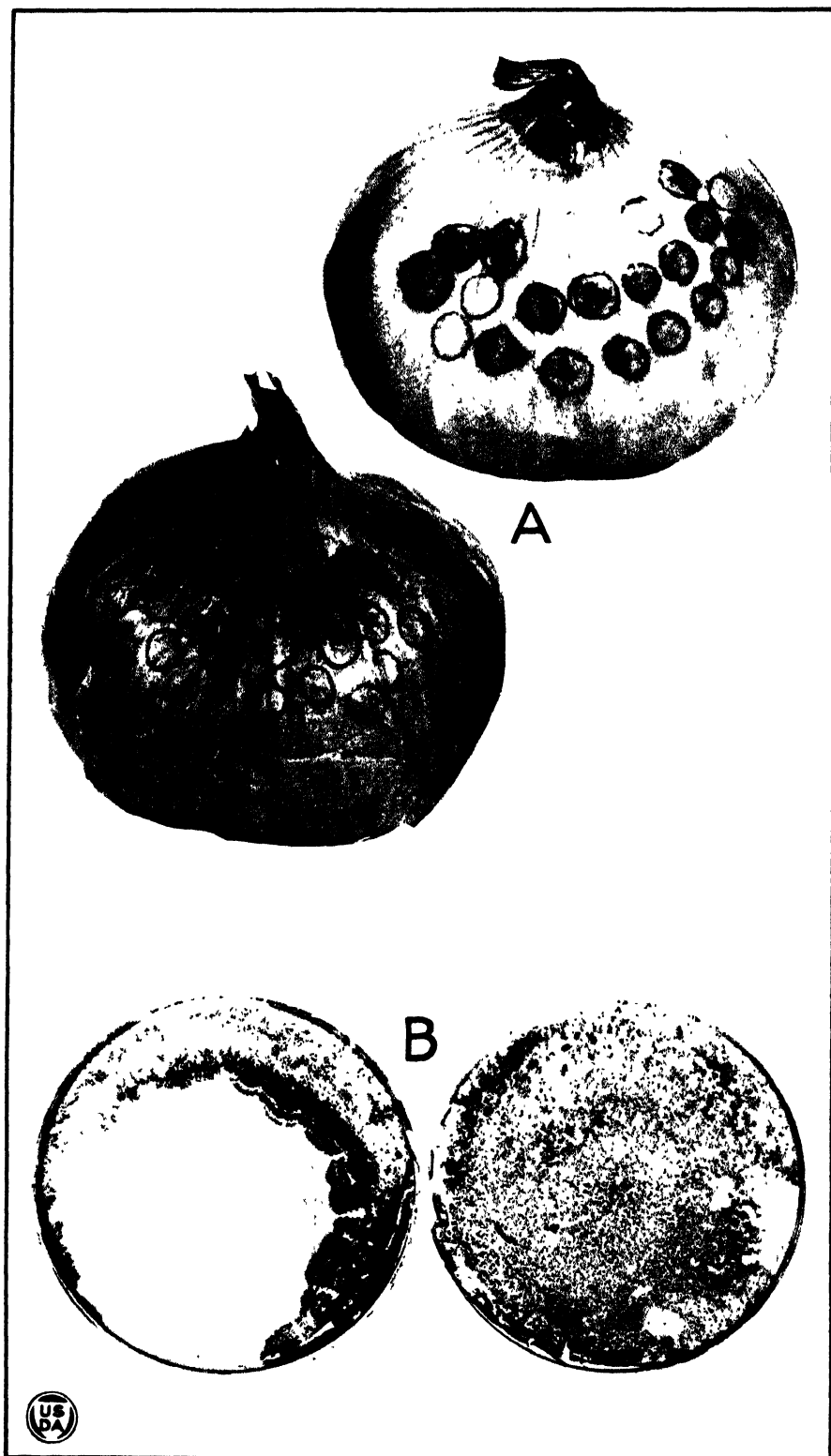
### PLATE 1

#### Varietal resistance to onion smudge.

White, yellow, and red onions grown in the same field of smudge-infested soil and gathered shortly before harvest. In the case of the white (susceptible) variety the fungus has already developed abundantly on the outer scale of bulb and neck. In the red and the yellow (resistant) varieties, the colored outer scales have remained free from the disease; infection has occurred only at the neck, where little or no pigment developed.

(1040)





## PLATE 2

Relation of volatile oil and scale pigment to the onion smudge organism.

A.—Relation of outer colored scale to infection: Two bulbs of Yellow Globe (resistant) variety inoculated by placing drops of spore suspension, in onion decoction, at points indicated by black circles.

Left: Inoculations made on dry, highly pigmented outer scale; no infection resulted.

Right: The thin, dry, outer pigmented scales were removed from this bulb and the inoculations were made on the first fleshy scale, which contained relatively little pigment; scale infection resulted in a majority of cases.

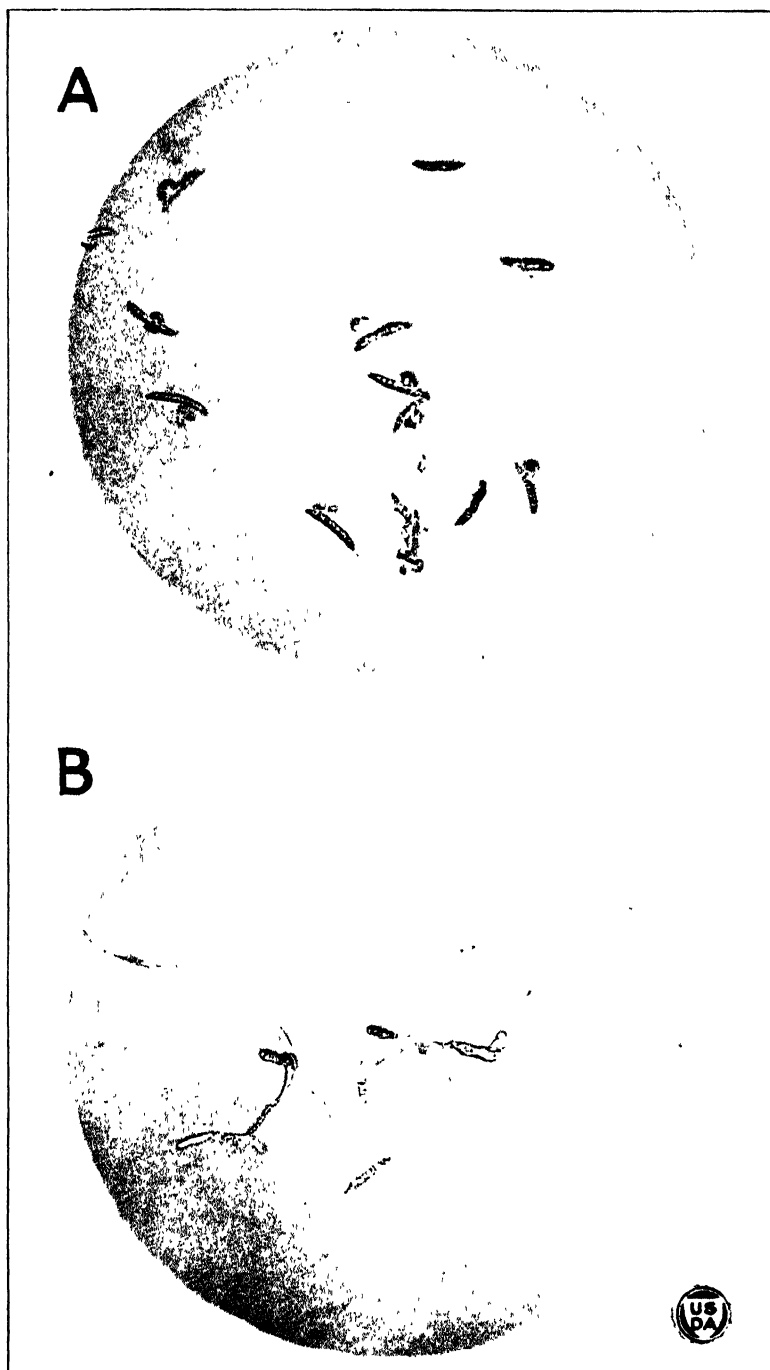
B.—Effect of volatile oil upon spore germination: Petri dish cultures prepared by pouring melted potato agar in which spores of the smudge organism were suspended. Plate at left exposed to volatile oil of onion by being inverted over a small amount of expressed onion juice for several days. The spores were all killed except those around the outer edge of the plate. As the juice deteriorated due to bacterial contamination, growth progressed inward from the unaffected area. Plate at right untreated; normal germination and growth occurred throughout.

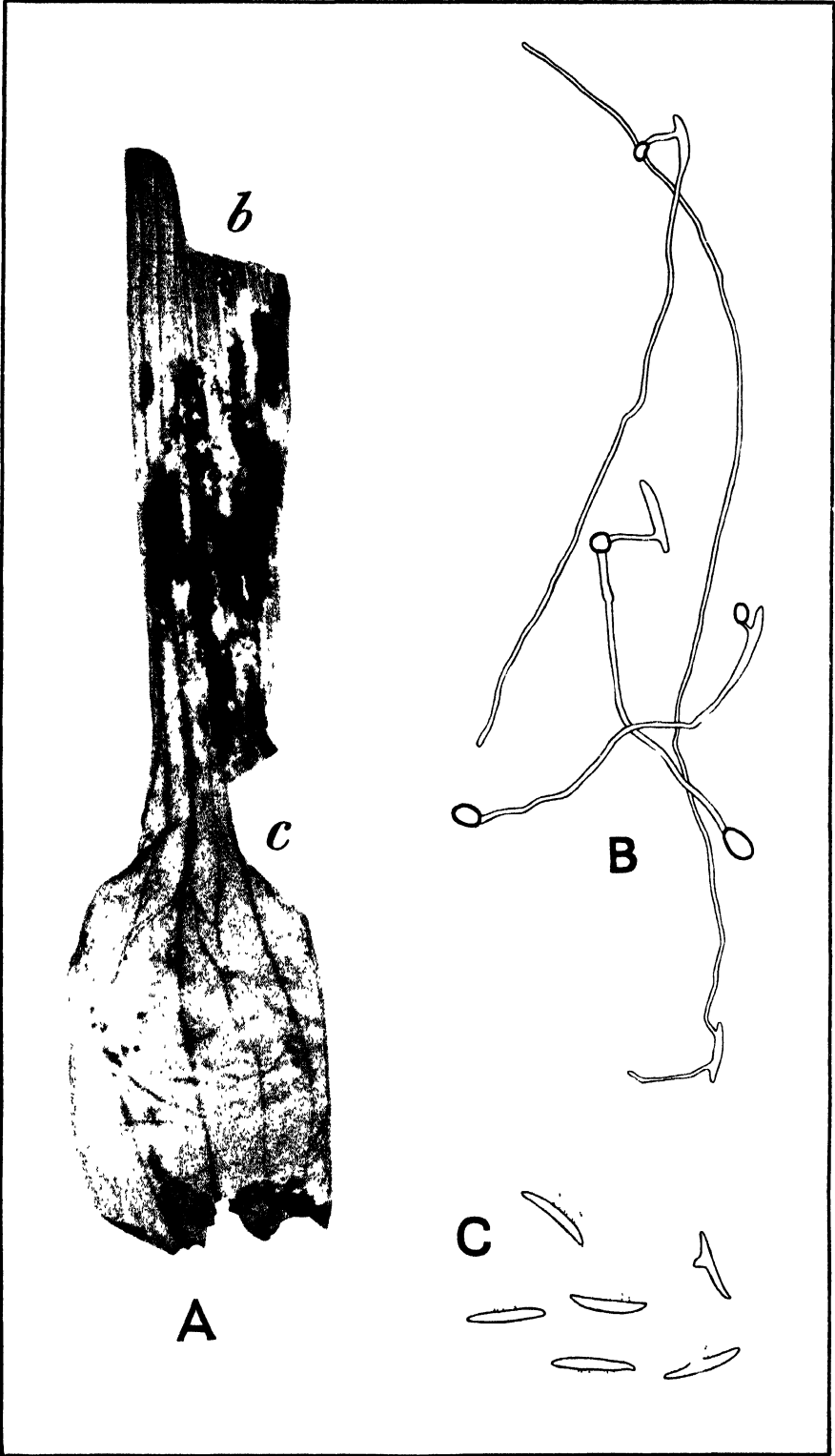
### PLATE 3

Effect of cold water extract from dry outer onion scales upon spore germination of *Colletotrichum circinans*.

A.—Photomicrograph of spores about 17 hours after being placed in an extract from the yellow scales. Note the masses of naked cytoplasm which have exuded from the spores after the tip of the young germ tubes have ruptured. Compare with B.

B.—Photomicrograph of spores about 17 hours after being placed in an extract from white scales. Note the normal germ tubes and appressoria. Compare with A. See also figure 1 and further explanation in the text.





#### PLATE 4

Correlation of pigment with onion smudge infection and with the occurrence of the toxic entity.

A.—Portion of outer scale removed at harvest time from a red onion set similar to that shown in Plate 2. (Enlarged  $\times 3$ .) Note that heavy smudge infection occurred at the neck above the point where pigment developed, while below that point the scale remained free from the disease. The scale tissue in the respective portions was tested for the presence of the toxic entity. Results of spore germination tests are shown in B and C.

B.—Normal germination resulting when bits of uncolored portion of scale from *b* in A were added to a drop of distilled water in which spores were suspended.

C.—Abnormal spore germination similar to that shown in figure 1, resulting when bits of the colored portion (*c*) of the same scale were placed in another drop of spore suspension. Note "rupturing" of germ tubes or production of short tubes of abnormally large diameter.





# THE EFFECT OF RESPIRATION UPON THE PROTEIN PERCENTAGE OF WHEAT, OATS, AND BARLEY<sup>1</sup>

By F. W. MCGINNIS, *Assistant Professor of Farm Crops, Division of Agronomy and Farm Management*, and G. S. TAYLOR, *Analyst, Division of Chemistry, Department of Agriculture, University of Minnesota*

## INTRODUCTION

Scientific literature contains considerable information dealing with the protein content of small grains. In a review of the available information bearing upon this subject, which has accumulated during the past century, one chief point of interest seems to be the variation in protein percentage found in the different grains when produced under different conditions of soil and climate.

Much study and investigational work has been conducted to ascertain the cause of the variation found in this regard, especially with wheat. Wheat has been the subject of greatest study, because of the interest of those engaged in the manufacture of wheat products, where the chemistry of the grain is of paramount importance in determining the quality of the output.

The reason why grain produced under a given environment will yield a berry entirely different in chemical structure from that produced under a condition of a different nature, has been a subject of much study with the grain chemist as well as with those engaged in physiological investigations.

It seems to be the generally accepted idea at the present time that the percentage of protein in wheat is due to a certain environmental condition which prevails during the growth and maturity of the grain. Upon analysis of the grain, grown under widely different environmental conditions, this is generally found to be true. It is common knowledge that wheats grown in the north central section of the United States are of a higher protein percentage and milling value than those grown in the eastern or far western sections. There is no doubt that the varieties being grown for the highest total yield in these respective areas, account, to a certain degree, for the difference in protein percentage in the grain. But when consideration is confined within a variety, the variation is found to be very wide.

Some investigators have attributed the difference to the available nitrogen in the soil, others to varietal differences, while most students of the subject have recognized it to be a physicochemical phenomenon correlated more directly with the climatic conditions which prevail during the life processes. In general, the protein percentage has been found to be dependent somewhat upon the length of the growing season and to a greater degree upon the length of the ripening period. As these two factors are dependent largely upon the amount of available moisture in the soil, the strongest correlation has been found between the amount of rainfall, its distribution, and the protein percentage.<sup>2</sup>

<sup>1</sup> Accepted for publication Feb. 20, 1923. Published with the approval of the Director as paper 397 of the Journal Series of the Minnesota Agricultural Experiment Station.

<sup>2</sup> MCGINNIS, F. W. THE RELATIVE EFFECT OF ENVIRONMENT UPON THE CHEMICAL COMPOSITION OF WHEAT, OATS, BARLEY, AND CORN. Unpublished thesis. Copies on file in Cornell University Library, Ithaca, N. Y. 1918.

That the external environmental conditions are responsible largely for the protein percentage found in the grain has been noted from the earliest observations. A realization, however, of the manner in which the contents of the grain are influenced by the capricious nature of external conditions and the physiological complexities which may arise during the life of the plant, is of more recent discovery. In order to understand fully how an environmental condition may affect a variation in the chemistry of the grain, it is necessary to follow out, from the earliest stages, the development of a kernel and the processes through which it reaches maturity.

Early investigational work along this line consisted of chemical analyses of the grain being made shortly after the time of flowering or when the mold of the grain was first formed, and continuing progressively at short intervals until the grain was mature. The nitrogen percentage was found to be highest as the kernel mold was formed and became decreasingly less as the grain filled and approached maturity. These observations led investigators to assume that most, or practically all, of the nitrogenous material was taken from the soil and moved into the grain during the earlier stages of growth, and that the filling of the endosperm with starch or carbohydrate material was carried out in the later stages of growth. Thus, it was concluded that the percentage of protein would be influenced directly by the amount of carbohydrate produced and translocated during the filling stage. As the environmental conditions in most of the grain sections are favorable to maximum growth during the early stage of development, and very often too severe to permit later growth to proceed or the grain to be filled, this was the natural conclusion to be drawn.

More recent work by Brenchley (2)<sup>3</sup> of the Rothamsted Experiment Station, Thatcher (5, 6), of the Minnesota Agricultural Experiment Station, and others, has brought out some slightly different ideas on the subject. These investigators have shown that the greater amount of nitrogenous material of the wheat or barley kernel is not taken up during the early stage of growth, but that the nitrogen inflow is continuous until the grain has reached maturity. According to Brenchley the nitro-carbohydrate movement into the grain is in constant ratio during the entire period of kernel development. It has been suggested that the variation in protein content is not due to the failure of the plant to produce and translocate carbohydrate material at the later stages of growth, but that the percentage of nitrogen found in the kernel is determined by the amount of carbohydrate lost by respiration during the desiccation process throughout the ripening period.

The difference of opinion as to which process is most effective in determining the character of the grain contents suggested the necessity of obtaining definite data to verify the later idea, which appears from available information to be an unwarranted conclusion. The authors have conducted a project through the years 1920 and 1921 to determine the variation in protein in wheat, oats, and barley, as affected by respiration, and to show the relative variation in protein when the same grains are grown under widely different conditions.

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<sup>3</sup> Reference is made by number (*italic*) to "Literature cited," p. 1048.

## PLAN OF EXPERIMENT

Marquis wheat, Improved Ligowa oats, and Manchuria barley were grown in 17 localities in the United States during the years 1920 and 1921. The grains were grown at experiment stations and substations in eight States which represent extremes, so far as the quality of grain produced is concerned. The seed was sent to each station from University Farm, St. Paul, Minn., each year, so that the seed planted would be identical. Plantings were made at the regular planting time in the spring and harvested when the grain was mature. Samples of the crop in each instance were sent to University Farm, where nitrogen determinations were made for total protein.

During the summer of 1921, respiration studies were made upon these grains during the ripening or desiccation period at University Farm, to ascertain the amount of material lost during this time. The studies were started when the grains reached the dough stage, before the desiccation process of maturity had begun, and continued until complete maturity.

Each morning at 8 a. m. samples of head material of each of the grains were collected, placed in an air-tight container, and taken to the laboratory. Twenty-five grain samples were picked from the spike by hand and immediately placed in a respirometer. The grains were subjected for 2 hours to a temperature of 30° C., after which the carbon dioxid respired was swept through the modified Truog absorption tower and collected in saturated barium hydroxid solution. The carbon dioxid evolved in this time was calculated in milligrams by titration with *N/10* hydrochloric acid and computed into carbon dioxid lost per 100 gm. of dry matter in 24 hours.

Table I shows the results of this work. It should represent the maximum loss by respiration which could be expected under field conditions. A constant temperature of 30° C. would average the high rate of respiration during the heat of the day and the lower rate during the cool night time.

Table I shows the amount of carbon dioxid produced and the actual amount of carbohydrate material lost from the grain, per 100 gm. of dry matter, in the process of respiration throughout the 16-day ripening period. The amount of carbohydrate material lost was calculated from the data showing the carbon dioxid evolved. The evolution and estimation of carbon dioxid is probably the most accurate method of determining the loss in weight due to oxidation, and is intended to account for the entire collective activities of respiration. While the process of respiration is a complex one, and it is impossible to account for the exact changes which take place, the carbohydrate material lost can be very closely estimated by assuming the process in general to be oxidation of sugars and starch.

The oxidation process results in the formation of carbon dioxid and water according to the empirical formula,  $C_6H_{10}O_5 + O_{12} \rightarrow 6CO_2 + 5H_2O$ . One part of carbohydrate with a molecular weight of 162 when oxidized by free oxygen, results in the formation of 6 molecules of carbon dioxid with an equivalent weight of 264. From these figures it will be seen that the formation of 100 gm. of carbon dioxid will require 61.36 gm. of carbohydrate material. Thus, the amount of carbohydrate lost is calculated as 61.36 per cent of the carbon dioxid evolved. For wheat this gives a loss of 10.038 gm.; oats, 11.092 gm.; and barley, 15.12 gm. of carbohydrate per 100 gm. of dry matter in 16 days, as indicated in Table I.



It was the intention to show the relative variation between wheat, a naked grain, and oats and barley, to which the glumes adhere, in the amount of material lost. As oats and barley are apparently less variable in their protein percentage than wheat,<sup>4</sup> it was thought that the adhering glumes might be influential in inhibiting respiration and consequently account for this lesser fluctuation. This demonstration was made impossible through inability to obtain samples of uniform moisture content, which, no doubt, is largely responsible for the wide difference in the results.

#### EFFECT OF RESPIRATION UPON PROTEIN PERCENTAGES

The result of the loss of carbohydrate material as affecting a variation in the protein percentage can be ascertained by estimating the percentage of the grain which is subjected to respiration activities. The composition of the average grain is approximately 12.50 per cent protein, 2.50 per cent ash, and 85 per cent carbohydrate. Respiration being confined to the carbohydrate portion of the grain, it would require approximately 7 per cent variation in this material to effect a 1 per cent difference in the protein percentage. As the number of grams of carbohydrate loss, in this case, is based upon the 100 gm. dry basis, these same figures represent the loss in percentage. The protein will vary 1 per cent for each 7 per cent loss or gain in carbohydrate.

Calculating from the amount of carbohydrate material lost as given in Table I, it becomes apparent that there is a possible variation in wheat of 1.46 per cent, oats 1.62 per cent, and barley 2.2 per cent protein, due to respiration.

While these figures seem upon first thought to be definitely significant, it is not probable that respiration creates a variation in the protein content as wide as the above possibilities suggest. As indicated, considerable carbohydrate is lost during the ripening period, but fresh material is no doubt being moved into the grain until complete maturity, which is sufficient at least to counterbalance that which is lost. It must be recognized, however, that the amount of material being moved into the grain will be a variable factor and dependent largely upon the environmental conditions which prevail at that time. Brenchley (1) has shown that in the development of the barley grain the maximum dry weight of 1,000 gm. is reached when the moisture content is approximately 40 per cent, and that this dry weight remains practically constant and does not fall off during the desiccation process, while the moisture content is being lowered to 21 per cent. The fact that the weight of dry matter is not lowered indicates that up to the time of maturity considerable material is being moved into the grain to compensate for that lost by respiration.

More confirmation of this supposition is presented by Harlan (3) in his study of the development of the barley grain, where he has shown that deposits of dry matter in the kernel continue until very near the point of absolute ripeness. He concludes (4) from irrigation studies that plants are able to utilize water to the date of full maturity, with much increase in dry matter content.

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<sup>4</sup> McGINNESS, F. W. OP. CIT.

The fact that the movement into the grain is continuous while respiration activities are in operation makes it impossible to determine the exact effect of respiration upon the variation in protein percentage. Assuming that the possible variation in protein is as great as mentioned above, namely, 1.46 per cent in wheat, 1.62 per cent in oats, and 2.20 per cent in barley, this would not be sufficient to account for the wide variation in the percentage as found in grain when produced in different localities, as given in Table II.

Upon analysis of Table II, a very wide variation in protein percentage is found for the grains grown in different localities and also for different years when grown in the same locality. In the year 1921 wheat grown in the hard spring wheat section at Fargo, N. Dak., yielded 16.10 per cent protein, while the same variety at Puyallup, Wash., shows 9.98 per cent. Another extreme variation may be seen by examination of the results at Havre, Mont., showing a protein content of 18.24 per cent, and Waterville, Wash., 9.19 per cent in the year 1920. In the former case there is a variation of 6.12 per cent in this regard, and 9.05 per cent in the latter. It would not be possible to attribute these differences to the respiratory process alone, as the above results show a 1.46 per cent variation in the protein which can be due to this phenomenon.

The data for oats indicate a much greater uniformity in this respect than is found in wheat. While this uniformity is especially pronounced throughout the year 1920 for the different areas, there are several cases in 1921 which vary considerably. The oats produced at Moccasin, Mont., Moro, Oreg., and Umatilla, Oreg., have a protein content which will average 14.12 per cent. Those grown at Waterville and Puyallup, Wash., and Corvallis, Oreg., average approximately 11.32 per cent. The spread in oats is not so great as in wheat, but this fluctuation of 2.80 per cent is much greater than that caused by the effect of respiration.

While the loss of carbohydrate material from the barley grain is much greater than that from wheat or oats, owing probably to the higher moisture content, it is not sufficient to account for the protein variation where the grain is grown under different conditions. Barley grown at Fargo, N. Dak., in 1920, yields 16.06 per cent protein, while that produced at Corvallis, Oreg., yields only 11.11 per cent. This is a difference of 4.95 per cent, which is more than twice the difference which could be due to respiration. In 1920, 14.50 per cent barley was produced at Havre, Mont., and Logan, Utah, yields 11.11 per cent. Here, again, is a spread of 3.39 per cent in the protein content, or 54 per cent greater than that attributed to respiration.

In a number of the analyses of each of the grains, the differences in protein percentage falls within the respiration possibility. These smaller differences may or may not be due to respiration, depending largely upon the environmental conditions under which the grain is matured. It is quite possible that respiration is effective to a degree in shaping the percentage composition of the grains when grown in the areas as indicated in Table II, but it is also possible that respiration plays a rather unimportant rôle in this regard when the synthetic process which goes forward throughout the period of desiccation is taken into account.

The exact influence of respiration upon the protein percentage composition of the grain is yet to be determined, but there is no foundation for the general sweeping statement that the variation in composition is dependent upon the respiratory process.

TABLE II.—Physical and chemical composition of wheat, oats, and barley grown in 16 different localities in 1920-21

Crop and locality.	Weight 1,000 grains.		Cerneous.		Protein.	
	1920	1921	1920	1921	1920	1921
<b>Wheat:</b>	<i>Gm.</i>	<i>Gm.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Original seed . . . . .	34.20	34.90	38	80	12.80	12.57
Havre, Mont. . . . .	19.60	29.75	100.	100	18.24	18.91
Moccasin, Mont. . . . .	25.85	25.00	100	95	15.85	16.00
Fargo, N. Dak. . . . .	28.90	22.50	100	100	12.34	16.10
Morris, Minn. . . . .	18.25	24.05	100	100	14.68	15.01
St. Paul, Minn. . . . .	24.00	25.80	92	91	13.42	16.46
Duluth, Minn. . . . .	28.75	21.50	67	100	13.80	11.76
Grand Rapids, Minn. . . . .	27.80	17.80	100	100	12.43	17.79
North Platte, Nebr. . . . .	20.00	22.70	100	100	14.55	15.01
Lincoln, Nebr. . . . .	25.00	20.60	100	94	12.81	14.52
Garden City, Kans. . . . .	22.10	19.30	98	100	15.24	15.10
Moro, Oreg. . . . .	26.10	23.95	100	100	16.82	15.88
Umatilla, Oreg. . . . .	24.25	18.60	100	100	15.57	18.52
Waterville, Wash. . . . .	30.35	33.85	0	98	17.10	15.65
Puyallup, Wash. . . . .	38.80	38.55	22.5	23.0	9.19	9.98
Corvallis, Oreg. . . . .	33.08	34.17	75.0	81.0	12.40	10.47
Logan, Utah. . . . .	30.24	34.60	100	100	14.22	14.97
<b>Barley:</b>						
Original seed . . . . .	34.45	36.70			12.46	12.48
Havre, Mont. . . . .	19.00	31.35			14.50	11.21
Moccasin, Mont. . . . .	24.65	33.10			14.01	13.81
Fargo, N. Dak. . . . .	36.05	22.22			12.66	16.06
Morris, Minn. . . . .	25.10	33.70			11.08	13.13
St. Paul, Minn. . . . .	28.22	24.70			11.06	13.01
Duluth, Minn. . . . .	32.16	40.15			14.00	11.08
Grand Rapids, Minn. . . . .	33.75	27.15			11.92	12.42
North Platte, Nebr. . . . .	25.75	28.15			12.44	13.03
Lincoln, Nebr. . . . .	24.15	31.05			11.83	12.33
Garden City, Kans. . . . .	22.12	23.52			12.12	12.41
Moro, Oreg. . . . .	17.50	28.40			13.05	10.88
Umatilla, Oreg. . . . .	27.79	16.75			12.62	13.71
Waterville, Wash. . . . .	36.11	36.00			14.34	13.89
Puyallup, Wash. . . . .	33.75	37.45			10.70	11.88
Corvallis, Oreg. . . . .	33.25	38.75			11.14	11.11
Logan, Utah. . . . .	37.60	33.45			11.11	12.11
			<b>Hull.</b>			
<b>Oats:</b>						
Original seed . . . . .	26.20	29.90	29.70	27.32	11.90	11.77
Havre, Mont. . . . .	24.60	28.55	25.22	28.63	13.82	13.29
Moccasin, Mont. . . . .	26.45	23.25	25.00	25.76	13.67	14.20
Fargo, N. Dak. . . . .	30.36	30.00	23.12	26.12	11.11	12.40
Morris, Minn. . . . .	27.75	28.55	26.12	27.30	13.47	11.39
St. Paul, Minn. . . . .	25.40	21.45	26.70	32.29	11.96	12.23
Duluth, Minn. . . . .	30.30	22.70	28.74	28.20	11.35	12.22
Grand Rapids, Minn. . . . .	30.13	28.70	24.87	30.01	12.52	12.10
North Platte, Nebr. . . . .	27.05	19.00	28.03	22.00	12.25	12.26
Lincoln, Nebr. . . . .	27.70	21.00	25.56	21.00	11.78	13.94
Garden City, Kans. . . . .	28.80	21.40	28.40	24.60	11.36	12.15
Moro, Oreg. . . . .	24.65	27.30	29.36	30.02	13.69	14.05
Umatilla, Oreg. . . . .	26.65	28.10	34.21	25.90	12.21	14.23
Waterville, Wash. . . . .	27.60	31.80	27.64	30.66	12.25	11.31
Puyallup, Wash. . . . .	32.10	33.20	25.78	26.21	11.10	11.20
Corvallis, Oreg. . . . .	29.80	33.50	27.04	30.12	11.22	11.45
Logan, Utah. . . . .	28.95	33.05	27.48	23.82	11.39	12.04



## SUMMARY

(1) The loss of carbohydrate material during the ripening of the wheat, oats, and barley grains is considerable. The greater percentage of the loss occurring before the process of desiccation begins while the grain contains above 40 per cent moisture.

(2) The protein composition of wheat, oats, and barley is influenced to a marked degree by the loss of carbohydrate material during the ripening period. Factors other than respiration or in connection with the process contribute largely to the formation of high protein grains.

(3) Wheat is more variable in the protein percentage than barley or oats, and barley appears to be more variable than oats.

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